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Intraperitoneal and Port Site Infiltration of Ropivacaine With and Without Dexmedetomidine for Postoperative Analgesia After Laparoscopic Cholecystectomy: A Randomised Controlled Trial

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ABSTRACT

Use of intraperitoneal and port site infiltration of local anaesthetics has been used to reduce postoperative pain and need for different analgesic agents delivered from various routes following laparoscopic cholecystectomy. Ropivacaine and highly selective alpha 2-agonist dexmedetomidine are the newer agents which are being increasingly used for peri-operative analgesia. The present study compared intraperitoneal and port site infiltration of ropivacaine and dexmedetomidine for postoperative analgesia in patients undergoing laparoscopic cholecystectomy. Sixty patients scheduled for laparoscopic cholecystectomy were enrolled in this prospective randomized double blind controlled study and assigned to 2 groups ($n = 30$) to receive either 40 ml of 0.25% ropivacaine with 2 ml of normal (Group R) or 40 ml of 0.25% ropivacaine combined with 1µg/kg dexmedetomidine diluted in normal saline to 2 ml (Group RD) infiltrated intraperitoneally and at port site after gall bladder removal following laparoscopic cholecystectomy. Haemodynamic parameters, Visual Analogue Scale (VAS) score for pain assessment, total duration of analgesia, total requirement of analgesic in first 24 hours were recorded. Data obtained were complied and analyzed with appropriate tests. A value of < 0.05 was considered significant. Total duration of postoperative analgesia was higher in Group RD whereas, VAS score and total rescue analgesic requirement were significantly lower as compare to group R. There was no significant difference in haemodynamic parameters between the groups. The addition of dexmedetomidineas an adjuant along with ropivacaine for intraperitoneal and port site infiltration is an effective method of postoperative pain relief following laparoscopic cholecystectomy without significant adverse effects.

KEY WORDS: dexmedetomidine, intraperitoneal infiltration, laparoscopic cholecystectomy, port site infiltration, postoperative analgesia, ropivacaine

INTRODUCTION:

Postoperative pain is a self-limiting phenomenon but a pain free postoperative phase increases patient's co-opeartion and activity levels, leading to early recovery.[1]Laparoscopic Cholecystectomy is one of the most frequently performed elective surgical procedure due to its enumerous advantages like minimal tissue trauma, lesser bleeding and pain, shorter recovery time and decreased hospital stay thereby, leading to overall reduced healthcare costs.[3]

The components of pain following laparoscopic cholecystectomy include visceral pain due to peritoneal inflammation resulting from release of inflammatory mediators and stretching of the intra-abdominal cavity. Shoulder pain results from irritation of phrenic nerve endings present in the diaphragm due to over-stretching caused by pneumoperitoneum. Parietal pain is due to surgical incision for the insertion of laparoscopic ports which is much lesser in intensity by virtue of its smaller size.[5]The major component of pain is attributed to visceral pain with maximal intensity during the first 24 hours postoperatively and is exacerbated by coughing, respiratory movements and mobilization.[6]

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Multimodal strategies for analgesia including infiltration of wound with local anaesthetics, administration of systemic narcotics and NSAIDs, intravenous and epidural patient controlled analgesia (PCA) with different class of analgesics and local anaesthetics enhance pain relief and reduce the side effects in postoperative period.[7,8,9]

Local anesthetics have been used intraperitoneally since 1950 as they are known to block the visceral afferent receptors signaling to the brain and potentially modify their conduction, thereby providing analgesia.[10,11,12] They also inhibit nociception through their action on nerve membrane associated proteins, inhibiting the release of prostaglandins and lysosomal enzymes along with leukocyte migration. The main advantage of using intraperitoneal route is that it does not have the adverse effects as of systemically administered drugs such as postoperative sedation, nausea and respiratory suppression because they act directly on the affected tissue. Recently, a variety of non-opioid adjuvant have been used clinically to speed up the onset and increase the duration of sensory and motor blockade.

The present study aimed at comparing ropivacaine with dexmedetomidine and ropivacaine as agents for pain relief by intraperitoneal and post site infiltration of local anesthetics in patients after laparoscopic cholecystectomy. The aim of the study was to determine the duration of postoperative analgesia of 0.25% ropivacaine with and without dexmedetomidine (1μg/kg) after intraperitoneal instillation in patients undergoing laparoscopic cholecystectomy. Hemodynamic changes, need of rescue analgesics and any side effects or complications were also recorded and compared.

MATERIALS AND METHODS:

After institutional ethical committee (IEC) approval, this prospective, randomized, double-blind study was conducted among 60 patients of ASA physical status I and II between age group 18–65 years of either gender scheduled for laparoscopic cholecystectomy under general anesthesia in BPSGMC (W), Khanpur, Kalan Sonepat, Haryana.

Cases with morbid obesity, significant systemic illness, known allergy to drugs under study, pregnant and lactating women, chronic pain syndrome and coagulation disorders, previous abdominal surgery, intra-abdominal drain placement postoperatively and refusal to participate were excluded from the study.

Based on our pilot study, taking a difference in duration of postoperative pain relief of 2 hrs between the two groups as clinically significant, to have an 80% power in present study with 95% confidence interval, 30 cases were recruited in each study group. A total of 60 patients were randomly divided into two groups of 30 each using computer generated simple random number table to receive either 40 ml of 0.25% ropivacaine with 2 ml of normal saline (Group R) or 40 ml of 0.25% ropivacaine plus 1μg/kg dexmedetomidine diluted to 2 ml with normal saline (Group RD).

Data was recorded in pretested performa meeting the objectives of the study. Pre-anaesthetic check-up (PAC) was done for each patient and written informed consent was taken. On arrival to the operating room, routine ASA standard monitors were attached. Baseline vital parameters were recorded prior to premedication, at every 5-minutes interval from the start of induction for first 30 minutes and then at every 10 minutes interval till the end of surgery.

Surgery was carried out under general anaesthesia with standard four trocar technique after pre-medicating with midazolam (0.04mg/kg), ondansetran (0.1mg/kg) and glycopyrrolate (4μg/kg) intravenously. A standard technique of general anaesthesia was followed for all the patients among both the groups. Pre-oxygenation with 100% oxygen was done for 3 minutes. Induction of anaesthesia was done with i.v. fentanyl (2 μg/kg) and propofol (2 mg/kg) followed by vecuronium bromide (0.12 mg/kg). The airway was secured using appropriate sized supraglottic airway device or with cuffed orotracheal tube of appropriate size and thereafter, nasogastric tube was inserted. Anaesthesia was maintained with 50% N_2O in oxygen with sevoflurane. Intermittent boluses of i.v. vecuronium bromide (0.02 mg/kg) were given to maintain neuromuscular blockade. Minute ventilation was adjusted to maintain end tidal carbon-dioxide [EtCO2] 40 ± 5 mmHg. Patients were placed in 15-20⁰ reverse Trendelenburg's position. During laparoscopy, intra-abdominal pressure was maintained at 10-12 mmHg. The study drug was prepared by the first investigator who was aware of group allocation of the cases and not involved in the study. The second investigator was unaware of the drug solution prepared by the first investigator and provided the surgeon with the drug for instillation and observed the patient until the end of study. At the end of surgery, a total of 32 ml of study drug solution was instilled intraperitoneally after peritoneal wash and suctioning, before removal of trocar (16 ml of solution was splayed over right

subdiaphragmatic space and another 16 ml in gall bladder fossa via epigastric port site) in Trendelenburg's position (15-20°).The remaining 10 ml of drug solution was infiltrated at trocar insertion sites after removal of trocar. Patients were kept in same position for 10 minutes to facilitate the dispersion of the drug solution at desired site.

Residual muscle paralysis was reversed with i.v. neostigmine (0.05mg/kg) and glycopyrrolate (0.01mg/kg). After suctioning, the nasogastric tube was removed and once the criteria for extubation was fulfilled, the airway device was also removed. In the post-anaesthesia care unit (PACU) no analgesic supplement was given till the patient complained of pain (VAS score <3). Diclofenac sodium 1.5mg/kg was be given intravenously as first line rescue analgesic if patient had VAS score >3 and i.v. tramadol 1 mg/kg was administered to any patient who still had VAS score >3 after 20 min of administration of first line analgesic.

Postoperatively, patients were assessed at 0-hour interval (immediate postoperative period), 1-hour interval (in the recovery room) and at the time when VAS>3 or at the time of demand for first analgesic for intensity of pain (irrespective of location and type of pain) utilizing VAS along with haemodynamic parameters (HR, SBP, DBP, MBP). The total analgesic consumption in the first 24 hours postoperatively was also calculated. The patients were monitored for side effects and complications of technique and drugs throughout intraoperative and postoperative period in both the groups, if any.

The data was entered in Microsoft Excel spread sheet and analyzed using SPSS software version 22.For quantitative data mean ± SD was calculated. For qualitative data chi-square test was used and percentage was calculated. For qualitative data repeated measure ANOVA and Post –Hoc Bonferroni test was used to find out mean difference between two groups. p-value <0.05 was considered as statistically significant.

RESULTS:

In the present study, both the groups were comparable with respect to mean age, sex, weight of the patients, and ASA distribution (Table 1). Comparison of intraoperative haemodynamic parameters (mean HR, SBP, DBP, MBP, SpO_2 and EtCO2) did not reveal any statistically significant difference amongst both the groups.

The haemodynamic parameters(HR, SBP, DBP, and MBP and SpO_2) were recorded postoperatively in both the groups at 0-hr, 1-hr, at the hour when

Table 1: Demographic distribution amongst the study groups

Variable	Group	Mean SD	p-value
Age (years)	R	35.73±10.68	0.561
	RD	34.20±9.61	
Weight (kg)	R	55.93±8.94	0.551
	RD	55.77±5.77	
Height (cm)	R	154.83±5.77	0.609
	RD	154.17±4.08	
BMI (kg/m²)	R	23.5±3.28	0.491
	RD	23.08±2.08	

VAS>3 or at the demand for first analgesic dose and found to be statistically insignificant.

The mean VAS score was recorded at defined time intervals and found to be lower in Group RD in comparison to Group R. The mean VAS score reading at 1-hour was statistically significant with $p = 0.043$, (Table 2).

Mean time for first rescue analgesic and the total duration of analgesia were significantly higher in cases administered with ropivacaine and dexmedetomidine as compared to ropivacaine alone with difference being statistically significant (p-value <0.001). Mean dose of rescue analgesic required was also higher in R group (p<0.001) (Graph 1).

Complications were noted in <20% of the patients in both the groups which were managed conservatively with no other life threatening adverse effects (Graph 2).

DISCUSSION:

Numerous studies available in literature have compared either one of the commonly used local anaesthetics like bupivacaine, lignocaine with a placebo such as normal saline or with different concentrations of same local anaesthetics.
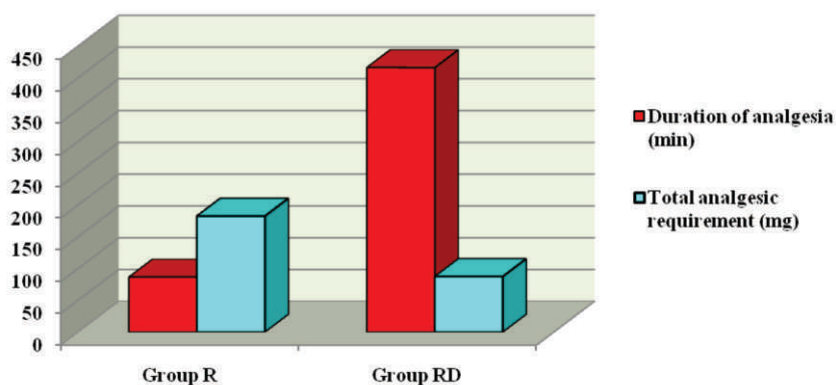
Ropivacaine in a concentration of 0.25% was chosen for this trial as higher concentrations of drug like 0.50% and 0.75% had already been studied enumerously and concluded that lower concentration is equally effective for providing postoperative analgesia when compared to higher drug concentrations[13,14]. Babu R et al. conducted a study including 60

Table 2: Comparison of mean VAS in the postoperative period amongst the study groups.

Time Interval	Group R (Mean ± SD)	Group RD (Mean ± SD)	p-value
0-hour	0.80 ±0.61	0.73 ±0.52	0.651
1-hour	1.72 ±0.34	1.53 ±0.37	0.043
Time for VAS>3	3.86 ±0.51	3.86 ±0.51	1.00

Table 3: Mean time for first rescue, and total number of rescue analgesics in 24 hours.

	Group	Mean±SD	Median	p-value
Duration of analgesia (min)	R	86.60 ±28.76	91.00	<0.001
	RD	416.20 ±127.36	454.50	
Total analgesic requirement(mg)	R	182.50 ±37.80	150.00	<0.001
	RD	87.50 ±39.80	75.00	

Graph 1: Bar diagram for comparison of Mean time for first rescue analgesic and total dose of rescue analgesia required in 1st 24 hour.

Graph 2: Bar diagram showing incidence of postoperative complications amongst the groups

patients for evaluation of postoperative analgesia following laparoscopic cholecystectomy after intraperitoneal instillation of 0.2% ropivacaine and 0.25% bupivacaine. It was concluded that both ropivacaine and bupivacaine are equally effective as postoperative analgesics but ropivacaine has better cardiac safety profile and is an attractive alternate to the bupivacaine[15].

Also, the volume of the drug solution selected for our study was 42 ml as many studies had already been conducted with higher volume of drug solutions like 50 ml, 75 ml, 100 ml and even up to 500 ml.[16] In a study conducted by Shivhare P, et al 30 ml of 0.5% ropivacaine and normal saline each was given to see the effect of intraperitoneal instillation of ropivacaine on postoperative abdominal and shoulder pain. Intraperitoneal instillation of ropivacaine was found to be an effective method of postoperative pain relief in laparoscopic cholecystectomy with such a low volume of drug solution which in consonance with our study[17]. In a study conducted by Jain S, et al. peritoneal cavity was irrigated with 500 ml of drug solutions for postoperative analgesia in laparoscopic cholecystectomy. It was found that high-volume low-concentration of intraperitoneal bupivacaine significantly increases postoperative duration of analgesia and reduces opioid requirement after laparoscopic cholecystectomy. However, the volume of the drug solution chosen for the study was comparatively higher and is in discordance with our study[16].

We used the intraperitoneal instillation of drug solution at the end of the procedure in the trendelenburg position with trocars intact along with infiltration of drug solution at the port site[18,19]. This dual technique of providing analgesia covers not only visceral pain arising from the gall bladder fossa but also parietal pain arising from the surgical trauma to abdominal wall. This avoids any bias in evaluation of pain and is also more comforting to the patient. Trendelenburg position aids in the flow and accumulation of drug solution over the coeliac plexus and free nerve endings leading to improved pain relief by blocking the nociceptive impulses.

All the haemodynamic parameters like mean heart rate, mean SBP, mean DBP, mean MAP, mean SpO2 and mean EtCo2 amongst both the study groups were comparable at the baseline, during intraoperative and the post operative period (p value>0.05). The results of our study are comparable to the study done by Meena R, et al comparing the efficacy of intraperitoneal infiltration of 0.5% bupivacaine and

0.75% ropivacaine to reduce postoperative pain in the patients with laparoscopic cholecystectomy. No significant difference (p>0.05) in haemodynamic characteristics were observed in concordance with our present study[20]. Similar finding were observed in the studies conducted by Sharan R, et al, Singh A et al [21,22].

In our study, mean VAS score was compared at 0-hour (immediate postoperative period), 1-hour postoperatively and at the hour when patient demanded of analgesia or VAS >3. The mean value of VAS score was overall low at 0-hour among both the groups and was statistically insignificant (p-value> 0.05). Mean VAS score at 1-hour postoperatively wassignificantly lower among group RD as compared to the group R (1.53±0.37 v/s 1.72±0.34). When the mean VAS score was >3 and the patient complained of pain they were administered with rescue analgesics as per protocol. So, the next recordings of VAS were comparable as these were recorded at different time intervals. These results are comparable to the studies done by Chiruvella S, Babu R and Oza VP[23, 14 & 24], which also recorded reduction in mean VAS in 24 hours.

But on contrary Gurusamy KS found that there was very low quality evidence that it reduces pain in low anaesthetic risk patients undergoing elective laparoscopic cholecystectomy[25].

The mean total duration of analgesia was significantly higher in cases administered with ropivacaine and dexmedetomidine as compared to ropivacaine alone group (416.20 min v/s 86.60 min; p-value <0.01) whereas, cumulative requirement of rescue analgesics in the postoperative period was lower in first 24 hour (182.50 mg v/s 87.50 mg; p-value<0.01) (Table 3). Our results are in congruence with studies done by Chiruvella S, Shukla U and Singh A, [23, 26 & 22] which also showed significantly better analgesic effect of dexmedetomidine when added to local an aesthetic solution.

In our study complications were noted in less than 20% of the patients among both the groups. Nausea and vomiting was observed in 5 patients of Group R and 6 patients of Group RD. Shoulder pain was observed in 2 patients among group R and RD. There was no episode of hypotension, bradycardia, arrhythmia, sedation, respiratory depression and any other life threatening side effects among both the groups. All the readings were comparable and the difference was found to be non significant in the two groups (p value>0.05).

The results were also in concordance with Singh A, Shukla U and Meena R observed no

statistically significant difference in regard to the adverse effects amongst the study groups (p>0.05) [22,26 &20].

CONCLUSION:

Our study concluded that combination of ropivacaine with dexmedetomidine when administered intraperitoneally and infiltrated at port site is an effective and reliable technique for postoperative pain management in the patients undergoing laparoscopic cholecystectomy under general anaesthesia. It not only reduces cumulative dose of rescue analgesics in the first 24 hours postoperatively but also improves the quality of analgesia. Moreover, this technique is not associated with any major adverse effects and may be incorporated in routine anaesthesia practice for providing postoperative analgesia in patients undergoing laparoscopic cholecystectomy.

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Prevalence of Obstructive Sleep Apnea in Hypertension Clinic Attendees of a Tertiary Care Centre

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ABSTRACT

Obstructive Sleep Apnea (OSA) and essential hypertension (HT) are common conditions affecting middle-aged and elderly adults. This study was done to know the percentage of hypertensive patients having Obstructive Sleep Apnea (OSA) and to correlate severity of OSA with the severity of hypertension. Fifty hypertensive patients attending hypertension clinic of People's Hospital enrolled as per inclusion criteria were subjected to detailed history, general examination, blood pressure measurements and overnight Polysomnography (PSG). Apart from this, clinical data gathered was applied to Sleep Questionnaires- Berlin Questionnaire, STOP-Bang Questionnaire, and NoSAS score. Mild and severe obstructive sleep apnea was found in 12% of patients each, whereas moderate obstructive sleep apnea was seen in only 6% of patients. 11 patients with stage I hypertension and 24 patients with stage II hypertension did not have OSA. Patients with stage I hypertension had mild OSA. Patients with stage II hypertension had all severity of OSA but there were more patients with severe OSA (6) then mild OSA (3) or moderate OSA (3). Only a minority of patients (30%) with essential hypertension had OSA. Majority of the patients with stage I and stage II hypertension did not have OSA. Severity of OSA increased with the increase in stage of hypertension but the observed correlation was statistically insignificant (p>0.05)

KEY WORDS: essential hypertension, NoSAS, obstructive sleep apnea, sensitivity

INTRODUCTION:

Obstructive Sleep Apnea (OSA) and essential hypertension (HT) are common conditions affecting middle-aged and elderly adults. The highest level of epidemiologic evidence supports the association between OSA and HT.[1,2]

Obstructive Sleep Apnea (OSA) is the most common form of sleep-disordered breathing.[3] It is a challenge for the healthcare system due to its high prevalence in the adult population and its linked morbidity and mortality (e.g., traffic accidents and cardiovascular complications).[4,5] Observational studies have shown that the prevalence of OSA is around 30% in hypertensive patients and nearly 80% in resistant hypertensive patients[6-9] Conversely, the reported prevalence of HT in those with OSA ranged from 15 to 56%.[10]

We hypothesized that in people having essential hypertension, some might have OSA, previously undiagnosed due to lack of symptoms or may not have been diagnosed by consulting physician. This study was conducted to assess percentage of hypertensive patients having Obstructive Sleep Apnea (OSA) and to correlate severity of OSA with the severity of hypertension.

MATERIALS AND METHODS:

In a unicentric observational study done during January 2018 to June 2019, 264 hypertensive patients attended hypertension clinic of tertiary care hospital, at Bhanpur area of Bhopal. They were explained about the study protocol and sleep study. Only 90 patients consented for the study and these were enrolled. Forty out of 90 patients who had associated conditions like symptomatic heart failure, neuromuscular disease, known diagnosis of COPD or Asthma, hypoxemia were excluded. Thus, 50 hypertensive patients were subjected to PSG for the diagnosis of OSA. The inclusion criteria of the study was age >18 years, known case of hypertension and patients consenting for the study.

Detailed information regarding socio demographic variables such as age, gender and occupation were recorded and entered in questionnaire. All subjects were enquired about a history of HT, medications used, and other medical

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conditions. General examination of patients was done. Height, BMI, neck circumference, and waist circumference were measured using an inextensible tape. Blood pressure measurement (in mm of Hg) was recorded and classified according to the Joint National Committee (JNC)-7 criteria. Apart from this, detailed clinical history was taken and clinical data gathered was applied in Sleep Questionnaires- Berlin Questionnaire, STOP-Bang Questionnaire, and NoSAS score.

Fifty eligible patients were subjected to overnight polysomnography (using Philips Alice-6 Diagnostic System) for the detection of OSA. Raw data obtained was manually scored in30-second epochs for sleep staging using the American Academy of Sleep Medicine (2012) criteria.[11] OSA was defined by the presence of apnea-hypopnea index (AHI) 5 or greater. Patients were classified either as normal (AHI < 5/h),mild (AHI ≥ 5 and <15), moderate (AHI ≥ 15 and <30), or severe (AHI ≥30).

Data was compiled using MS Excel and analyzed using IBM SPSS software version 20.Frequency and percentage were calculated & statistical test (Chi-Square) was applied wherever applicable; $p < 0.05$ was taken as statically significant and $p < 0.01$ was taken highly significant. Sensitivity and specificity were calculated for a sleep questionnaire against AHI and expressed as a percentage and compared using the ROC curve.

RESULTS:

Fifty hypertensive patients were recruited for the study. Mean age of patients was 50years.Majority of the patients i.e. 74% were males. Maximum number of patients were obese. Grade 1, grade 2 and grade 3 obesity were observed in 34%, 32%, and 8% patients respectively. Demographic data and Baseline characteristics of hypertensive patients are depicted herein (Table 1).

Our study included the hypertensive patients. Majority of the patients were stage II hypertensives (72%) and Stage I hypertension was observed in 28 % of the patients. Maximum number of the hypertensive patients did not have Obstructive Sleep Apnea. Mild (AHI ≥5 - <15) and severe (AHI ≥30) obstructive sleep apnea was found in 12% of patients each. Moderate obstructive sleep apnea (AHI ≥15 - <30) was seen in only 6% of patients. Majority of the patients with stage I and stage II hypertension did not have OSA. Patients with stage I hypertension had mild OSA. In Patients with stage II hypertension ,severe OSA was more common than was found in mild or

moderate OSA. No significant association was found between hypertension and OSA ($p > 0.05$).(Table 2)

Our study observed positive correlation between severity of hypertension and AHI i.e. as the stage of hypertension increased, AHI also increased but the observed correlation was statistically insignificant ($p > 0.05$).(Table 3) PSG diagnosed OSA in 15 patients . Berlin, Stop Bang and NoSAS identified 10, 12 and 9 patients respectively as high risk for OSA. Similarly out of 35 patients diagnosed with no OSA by PSG, no risk for OSA was observed in 29 cases each with all the questionnaires. Most sensitive questionnaire for diagnosis of OSA was STOP-Bang (80%) followed by Berlin (66.7%) and NoSAS (60%). Similarly, specificity, PPV as well as NPV were also high for STOP Bang questionnaire as compared to other two questionnaire. Area under curve was maximum for STOP Bang questionnaire (0.81).(Table 4)

DISCUSSION:

In our study, 50 hypertensive patients having mild, moderate or severe HTN underwent Polysomnography. OSA was found in only 30% of the patients. In 15-18 patients detected to be at high risk for OSA by the three questionnaires, OSA was confirmed by PSG in 60-80 % of the patients. This reinforces that all hypertensives need not undergo PSG. Only those classified as high risk for OSA by any of the three questionnaires – Berlin, STOP-Bang and NoSAS should undergo PSG. Twenty eight percent of our patients had stage I hypertension,72% had stage II hypertension. There were no patients with resistant hypertension in our study.

Majority of the patients with stage I hypertension did not have OSA(78.6%). Only21.4% patients who had stage I hypertension had OSA, which was mild. Similarly, majority of the patients with stage II hypertension did not have OSA(66.7%) but there were higher number of patients with severe OSA(16.7%) than mild and moderate OSA(8.3% and 8.3% each) in this group. Stage II hypertensive patients had higher number of patients with OSA as compared to patients with stage I hypertension, but no association was found between stage of HTN and presence of OSA ($p > 0.05$). We observed that severity of OSA is not related to the severity of HTN.

Epidemiologic evidence supports the association between OSA and HT. OSA is one of the underlying relevant treatable factors associated with hypertension.[1,2] Previous studies have demonstrated the occurrence of hypertension in patients with Sleep apnea. However, the present study is unique as we

Table 1: Demographic data and Baseline characteristics of hypertensive patients (n=50).

Baseline Variables		Frequency (n=50)	Percentage (%)
Age Group (years)	<35	4	8
	35-44	10	20
	45-54	19	38
	55-64	11	22
	=65	6	12
Gender	Male	37	74
	Female	13	26

BMI	Frequency (n=50)	Percentage (%)
Underweight (<18)	2	4
Normal (18-22.4)	2	4
Overweight (22.5-24.9)	9	18
Grade 1 obese (25-29.9)	17	34
Grade 2 obese (30-39.9)	16	32
Grade 3 obese (=40)	4	8

Table 2: Association of OSA with hypertension.

OSA	Hypertension	
	Stage I n (%) , 14(28 %)	Stage II n (%), 36(72%)
None	11 (78.6)	24 (66.7)
Mild (AHI =5 - <15) (12%)	3 (21.4)	3 (8.3)
Moderate(AHI=15-<30)(6%)	0 (0)	3 (8.3)
Severe (=30) (12%)	0 (0)	6 (16.7)
Chi Square (?2)=95.12, p=0.16		

Table 3: Correlation of Stage of hypertension with AHI.

R	R Square	Adjusted R Square	SE of Estimate	F	Sig.
0.205	0.042	0.022	26.821	2.111	0.153

have tried to determine the occurrence of OSA in hypertensive patients. The mean age of hypertensive patients was 50.34±11.39 years and majority of the patients (74 %) were obese in our study. Grade 1, grade 2 and grade 3 obesity was observed in 34%, 32%, and 8%hypertensivepatients respectively. Studies in the past have already established that hypertension and OSA are associated with obesity.[12,13]

In 168 hypertensive patients studied by Borsini E et al, there were more patients with mild OSA(40.5%), than with moderate (23.8%) and severe OSA(20.2%).In contrast to our study, high prevalence of OSA in hypertensive patients was found by Borsini E et al as they performed PSG only in those hypertensive who had high risk for OSA as screened by ESS and STOP–Bang questionnaire.[14]Fletcher EC

Table 4: Diagnostic accuracy of sleep questionnaire.

	Sleep questionnaire		
	Berlin , n (%)	STOP-Bang, n (%)	NoSAS, n (%)
Proportion of high risk	16 (32.0)	18 (36.0)	15 (30.0)
OSA confirmed by PSG	30%		
Sensitivity	66.7	80	60
Specificity	82.9	82.9	82.9
PPV	62.5	66.7	60
NPV	85.3	90.6	82.9
AUC	0.75	0.81	0.71

et al studied 46 hypertensives and 34 normotensive-patients to know the proportion of patients having OSA. All patients underwent polysomnography. Proportion of hypertensive patients having OSA was similar to our study (30%). Disordered breathing events per hour were significantly higher in hypertensive patients (18.1) as compared to the normotensive controls (8.9) and the observed difference was statistically highly significant (p <0.002).[9] Udwadia et al conducted two-phase cross-sectional prevalence study and 250 patients underwent an overnight home sleep study. The estimated prevalence of SDB (apnea-hypopnea index of 5 or more) was 19.5%).[15] Borgel J et al evaluated hypertensives for unrecognized secondary causes of HTN.OSA was found in 70.8% of the screened patients.[16]

In our study, Berlin, STOP-Bang, and NoSAS scoring systems were used to assess the risk of OSA. Maximum patients with high risk of OSA were identified by the STOP-Bang questionnaire (18) followed by Berlin questionnaire (16) and NoSAS score (15).We compared the risk assessment of OSA among various questionnaires to confirm and compare their diagnostic validity. Out of 18 patients detected to have high risk of OSA by the STOP-Bang questionnaire, PSG detected OSA in 12 patients. Those at high risk of OSA by Berlin questionnaire (16 patients) and NoSAS score (15patients), PSG detected OSA in 10 patients and 9 patients respectively. The sensitivity was highest (80%) for STOP-Bang questionnaire and lowest (60%) for NoSAS score . The specificity was similar for all three questionnaires (82.9%). NPV and PPV were highest for the STOP-Bang questionnaire. The diagnostic accuracy of various questionnaires was assessed using the Receiver Operating curve (ROC). The area under the curve measures discrimination, that is, the ability of the test to correctly classify those with and without the disease or at high risk for the disease. The area under curve was highest for the STOP-Bang questionnaire i.e. (0.81) followed by the Berlin questionnaires and NoSAS score.

Talarowska P et al in there study observed that maximum number of high risk for OSA was identified by the NoSAS score (63.7%) followed by Berlin (41%) and STOP-Bang questionnaires (33.9%).[17] Tan A et al found that all three questionnaires performed similarly regardless of AHI cutoff values. The AUCs clusteredaround0.692–0.738 and 0.682–0.748 for AHI ≥20 and 30 events, respectively i.e. there were no differences in performance between the three questionnaires.[18] Tan A et al suggested that these questionnaires should be used only as screening tools to rule out low-risk subjects for severe OSA. Subjects who are identified as high risk via these questionnaires will still require further confirmation by PSG.[18]

The Berlin questionnaire takes a longer time to fill but the interpretation is simple. The scoring of the STOP-Bang questionnaire is simple and it was observed to be more sensitive than other questionnaires. The NoSAS score is slightly more complicated as it has a differential scoring system for each variable, but it is the easiest to fill. All the questionnaires can be used as a screening tool for identification of OSA but the best screening tool to diagnose the presence of OSA i.e. mild, moderate as well as severe OSA, was STOP-Bang questionnaire.

CONCLUSION:

Only a minority of patients (30%) with essential hypertension had OSA. Our study showed that all patients with essential hypertension do not

require PSG. Hypertensive patients should first be screened by any of the three questionnaires Berlin questionnaire, STOP-Bang questionnaire, or NoSAS score. Those found to be at high risk for OSA by questionnaires should be subjected to polysomno-graphy to rule out OSA. Patients with stage I hypertension had mild OSA. More patients with stage II hypertension had severe OSA than moderate and mild OSA.

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Prevalence of Potentially Malignant Disorders of the Oral Cavity in Bhopal

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ABSTRACT

Oral cancer is generally preceded by benign lesions for a varying length of time. Many of them show high potential to become cancers and therefore termed as 'precancerous'. Even though only a small proportion of precancer actually progresses to oral cancer, this development forms a source for over 70% of oral cancers in India. An epidemiological survey was conducted in the population of Bhopal city to estimate the prevalence of potentially malignant disorder of the oral cavity. Our study comprised of a total 2509 samples out of which males were 1354 (54%) and females were 1155 (46%).Out of these, 778 i.e. 31% of the total sample size were habit positive, either chewing tobacco, chewing areca, smoking tobacco, and drinking alcohol or combinations of above. This multi-center cross-sectional study revealed that the deleterious habits in males showed prevalence of 87% and in females showed prevalence of 13%. The ratio of male: female prevalence was determined to be 7:1. This high incidence of PMDOC calls for urgent need to address the issues of tobacco chewing and raise awareness about oral cancer.

KEYWORDS: habit index, malignant disorders, oral cavity, white lesions

INTRODUCTION:

Since time immemorial teeth, mouth and face have been intrinsically fascinating for mankind. They have been and will continue to be the subject of many oral and written beliefs, superstitions, traditions and an object of a wide range of decorative and mutilatory practices. At the same time they have been the cause of considerable sufferings for many. Recorded history is replete with descriptions of methods used by a range of ancient and relatively contemporary cultures to combat the symptoms and effects of disease affecting the teeth and other peri-oral structures[1].

In day-to-day clinical experience, the dental and general medical practitioners often encounter a wide spectrum of oral mucosal lesions. Globally, oral cancer constitutes one of the most common cancers with a very high incidence in the developing countries. In the Indian scenario, oral cancer is the second most common cancer. It ranges from innocuous mucosal alterations which may need simple therapeutic remedies and patient counselling to lesions of a life-threatening nature[1].

Oral cancer is generally preceded by some benign lesion for a varying length of time. Many of them show a high potential to become cancer and therefore termed as 'precancerous'. Even though only a small proportion of precancer actually progresses to oral cancer, this development forms a source for over 70% of oral cancers in India. Individuals with precancer, run a 69 times higher risk to develop oral cancer as compared to tobacco users who do not have precancer. The recognition and management of precancer therefore constitutes a vital oral cancer control measure.

In the review article of S. Warnakulasuriya et al in 2007[3], an international working group comprising of specialists in the fields of epidemiology, oral medicine and pathology and molecular biology with a special interest in oral cancer and precancer, met in London in May 2005. They discussed the current concepts, terminology, classifications, natural history, pathology and molecular markers to critically analyze the evolution of knowledge and practice concerning

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the diagnosis and management of what have been traditionally called as precancerous lesions and conditions of the oral mucosa. The workshop was coordinated by the WHO Collaborating Centre for Oral Cancer and Precancer in the UK. The working Group did not favour subdividing precancerinto lesions and conditions and the consensus was of the view to refer all clinical presentations that carry a risk of cancer under the term 'potentially malignant disorders' to reflect their widespread anatomical distribution[3].

Potentially malignant disorders of oral cavity (PMDOC) have shown to be commonly associated with the use of tobacco and areca nut in various forms. The high prevalence of these habits in the regional population of Bhopal needed the proposed study to know the prevalence of PMDOC in this region. This study was planned to increase the awareness about PMDOC among Bhopal population and to help various the authorities in planning preventive care to stop carcinogenesis from PMDOC.

An epidemiological survey was conducted in the population of Bhopal city with purpose (a) to estimate the prevalence of potentially malignant disorders of the oral cavity(b) to identify the prevalence of habits in malesand females in the study population; (c) to identify the prevalence of type of potentially malignant disorders in the study population and (d) to find out the association of habit index with the potentially malignant disorders. Cancer registry at Gandhi Medical College, Bhopal suggested that an estimated 6 lakh people use tobacco-in form of cigarettes or gutka or raw tobacco out of nearly 24 lakh plus population of Bhopal. One in four persons consumes tobacco on a regular basis in Bhopal. One in five of pre-cancer diagnosed case is of a teenager in Bhopal, Oral cavity cancer is increasing by 2.5% per year in the city[3].

MATERIALS AND METHODS:

According to Global Adult Tobacco Survey (GATS 2016-2017)[4] in India, 42.4% men, 14.2% women and about 4.0% minors (15–17 years) consumed tobacco. India has 28.6% adults who use tobacco and 3.4% adults who use both smoke and smokeless tobacco[4]. They also noted that 19% men and 2% women smoked and 29.6% men and 12.8% women used smokeless tobacco products. According to GATS 2016-17, in Madhya Pradesh, the percentage of consumption of smoked tobacco was 10.2% and smokeless tobacco was 28.1%. 52.2% of men, 17.3%of women and 34.2% of all adults consume either smoke or smokeless tobacco in Madhya Pradesh[4].

The prevalence of tobacco used by minors aged 15-17 years was found to be 13.1 % in MP. 24.7% of adults were found to be exposed to passive smoke at public place and 38% in offices[4].

The data from Madhya Pradesh voluntary health association has suggested that all the parameters related to tobacco abuse have critically increased (Table 1). This prompted us to go for a multi-cluster sampling procedure to focus on the data related to the deleterious and high risk habits and in turn correlate them with the life style and nutritional parameters. The importance of habit counselling at level one prevention of potentially malignant oral disorders has been well established now. All the data was collected through a structured questionnaire. The ethical committee clearance and consent was obtained from each participant as per the protocol mentioned by ICMR

Multi-cluster sampling was done with the help of public health dentistry and certain camps were organized in collaboration with Lion's Club and Rotary Club. The study also covered the slum areas of Bhopal. At each camp site education against tobacco, betel nut and alcohol abuse was conducted. Many of the engineering colleges were screened and since the students came to such colleges from different districts of Madhya Pradesh, the clustering effect and randomization added to the data.

A total of 2509 patients were screened of which 1354 were males and 1155 were females; of these 778 patients consumed tobacco, betel nut, alcohol etc or any combination of the above mentioned substances. After screening the patients for the various OPMD, they were also assessed for nutritional, chew, alcohol and smoke index. The nutritional index was based on BMI and each deleterious habit was denoted as 'chew index, smoke index and alcohol index'. The habit index was defined for the first time by Bailoor and Nagesh in 2005[5] and is a statistically sensitive indicator of the prognosis of an oral premalignant and malignant lesion.

HABIT INDEX:

It may be used by the dentists to quantify the effect of habit on oral mucosa and general health. For example, if a person smokes 10 cigarettes for the last

Table 1: Tobacco use in India and Madhya Pradesh.

	India (%)	Madhya Pradesh (%)
Tobacco users in any form	34.6	39.5
Smokers	14	16.9
Bidi smokers	9.2	13.4
Cigarette smoker	5.9	5.1
Chewable form of tobacco user	25.9	31.4
Persons getting affected by passive smoking on public places	29	40
Persons getting affected by passive smoking in public transport	17.5	34.1
Persons getting affected by passive smoking in offices	29.9	32

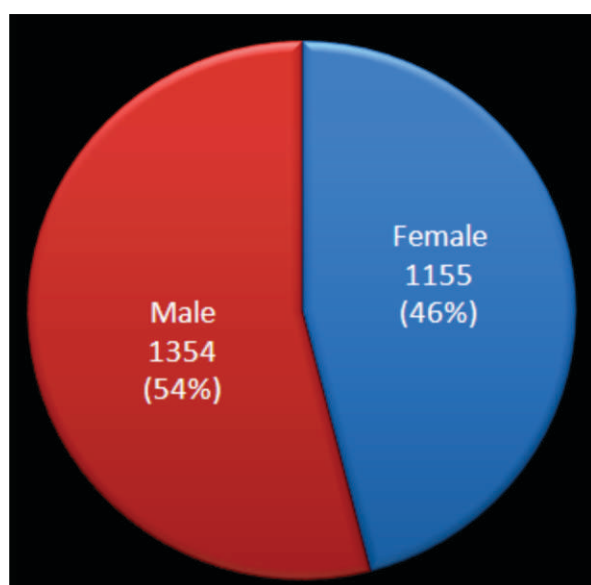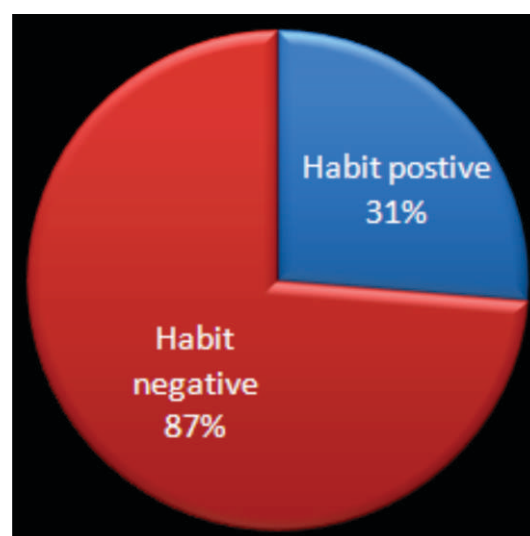15 years, then the **smoke index** will be 10 *15 =150.

Alcohol consumption is usually measured in peg per week x number of years, for example if a person consumes 2 pegs of whiskey a day / 14 pegs per week, for ten years then his **alcohol index** will be calculated 14 * 10 = 140.

Betel chewing, betel leaf chewing with slaked lime and catechu could also be quantified in similar fashion by a product of the frequency per day X no. of years. At the frequency of 8 a day for twelve years of betel **chew index** would be 12 * 8 = 96.

All the observations and data were tabulated and statistical analysis was performed using the software DECISION ANALYST STAT 2.0™

RESULTS:

Our study comprised of a total 2509 samples out of which, males were 1354 (54%) and females were 1155 (46%) (Graph1).

Graph 1: Gender-wise distribution of screened patients.

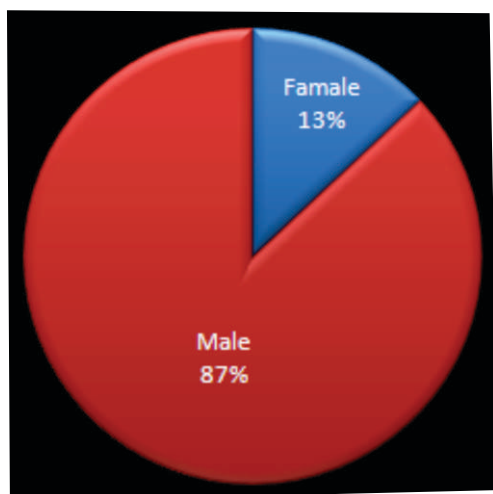**Graph 2**: Habit positive percentage in study population.

Out of these, 778 i.e. 31% of total sample size (Graph 2) were habit positive, either chewing tobacco, chewing areca, smoking tobacco, and drinking alcohol or combinations of above.

Out of these 778 habit positive cases, the habits were significantly higher in males; males (87%)and females(13%) (Graph 3).

218 cases were having the potentially malignant mucosal disorder which makes the percentage of 8.6% of total screened population and 28% from the habit positive cases (Graph 4).

Potentially malignant disorders of oral cavity in total sample considered were preleukoplakia, lichenoid reaction, leukoplakia, leukoedema, leukokeratosisnicotina palate, Oral Submucous Fibrosis. Percentage of each of the potentially malignant disorder from total screened population was preleukoplakia (0.2%), lichenoid reaction (0.3%), leukoplakia (1.3%), leukoedema (1.5%), leukokera-tosisnicotina palate (1.8%), Oral Submucous Fibrosis (4.74%) (Graph 5).

Graph 3: Gender-wise distribution of habit positive cases.

Graph 4: Percentage of mucosal disorders in total screened sample (A) and from the habit positive sample (B).

Mucosal lesions were more prevalent in males. Reason may be attributed to the more prevalence of tobacco habits in males. The gender specific percentage of OPMD was: preleukoplakia (M – 0.22%; F – 0.17%), lichenoid reaction (M – 0.5%; F – 0.3%), leukoplakia (M – 2%; F – 0.5%), leukoedema (M – 2.5%; F – 0.4%), Leukokeratosisnicotina palate (M – 3%; F – 0.4%), Oral Submucous Fibrosis (M – 7.4%; F – 1.5%) (Graph 6).

Leukoplakia was further divided into its types and its prevalence percentage was as follows: ulcerated (M – 0.14%; F – 0%), verrucous (M – 0.22%; F – 0.17%), speckled (M – 0.5%; F – 0.08%), homogenous (M – 0.88%; F – 0.25%) (Graph 7).

HABIT INDEX:

Association of habit index- chew, smoke and alcohol index with the occurrence of potentially malignant disorder was observed. For chew index, the mean habit index was as follows, OSMF - 53.8, leukoplakia – 41.08, preleukoplakia - 40.4, lichenoid reaction - 32.08, leukoedema – 24.8, Leukokeratosisnicotina palate – 22.4 (Graph 8a). For smoke index, the mean habit index was as follows, Leukokeratosisnicotina palate – 134.08, leukoplakia – 127.3, leukoedema – 108.7, OSMF – 18.45, preleukoplakia – 16, lichenoid reaction – 12 (Graph 8b). For alcohol index, the mean habit index was as follows, leukokeratosisnicotina palate – 104, lichenoid reaction – 84.37, OSMF – 73.4, leukoplakia – 67.3, Preleukoplakia – 53.4, leukoedema – 42 (Graph 8a)

DISCUSSION:

In India, tobacco consumption is responsible for half of all the cancers in men and a quarter of all the cancers in women, in addition to being a risk factor for cardiovascular diseases and chronic obstructive pulmonary diseases[6].
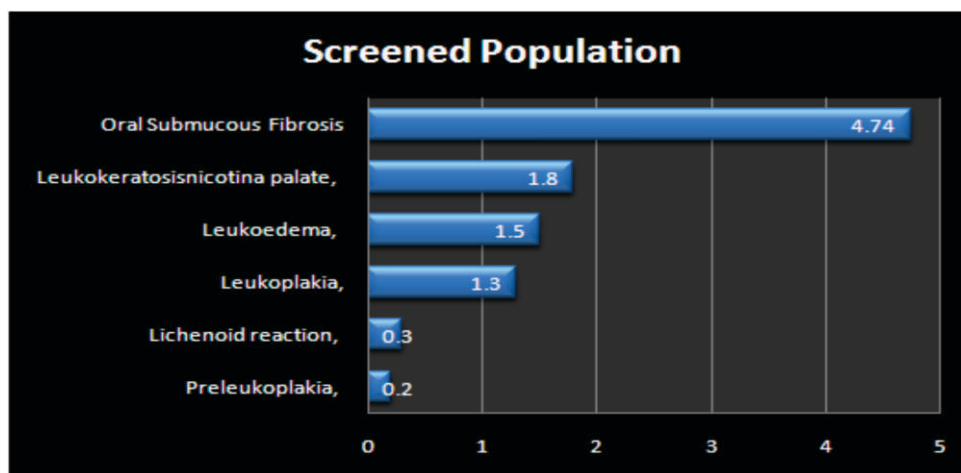
Tobacco is one of the legally available and most commonly used substance that kills one third to half the people who consumes it. As of year 2000, there were an estimated 1.1 billion smokers worldwide and this number is estimated to increase to 1.45 billion in 2020[6]. Deaths due to tobacco and other deleterious substances are likely to be more than double between years 1998 and 2030, when there may be more than 8 million deaths annually. This means that tobacco-related deaths will exceed the total number of deaths from malaria, maternal and major childhood conditions, and tuberculosis combined[6].

Certain forms of smoked (*bidis* and *kreteks*) and smokeless (chewing) tobacco are most prevalent in countries of South-East Asia. The World Health Organization predicts that tobacco deaths in India may exceed 1.5 million annually by 2020[9]. Tobacco use, a man-made epidemic, kills about 5.4 million people a year globally[7].

The chewing habit is seen in high school students, labourers and also blue collar workers. This worrisome distribution of habit is a harbinger of the coming epidemic of Oral Cancer. The socio economic status is no longer distinctive or accurate in predicting the use of Ghutka.

Many large-scale cohort studies have been carried out on tobacco users in different states of India right from the 1960s. In urban study areas, among males aged 25–69 years nearly 60% of all those who died were smokers as compared to 39% among controls. Thus, middle-aged smokers had significantly higher death rates than non-smokers from all medical causes combined. The risk of death due to tuberculosis –

Graph 5: Prevalence of oral potentially malignant disorder in total screened population.

Graph 6: Gender specific prevalence of oral potentially malignant disorder in total screened population.

Graph 7: Gender specific prevalence of types of leukoplakia in total screened population.

Graph 8a: Mean habit for chew index.

Graph 8b: Mean habit for smoke index.

Graph 8c: Mean habit for alcohol index.

was 6.3 times higher, other respiratory illnesses 3.7 times higher, vascular causes 1.7 times higher and neoplastic causes twice as high among smokers tha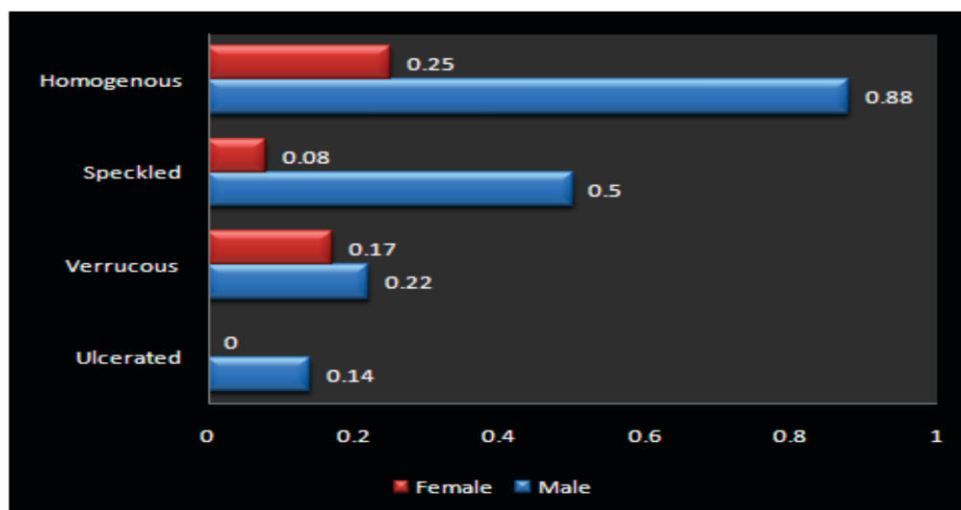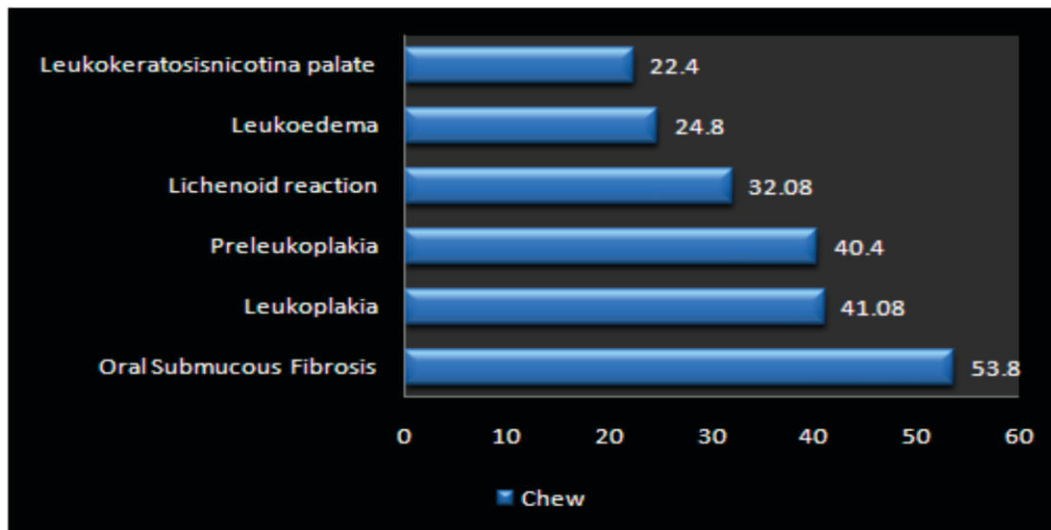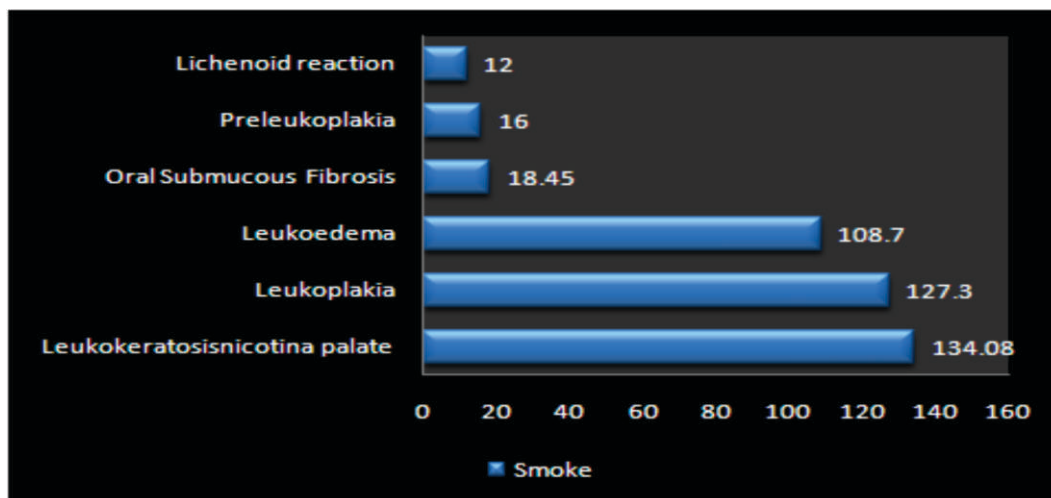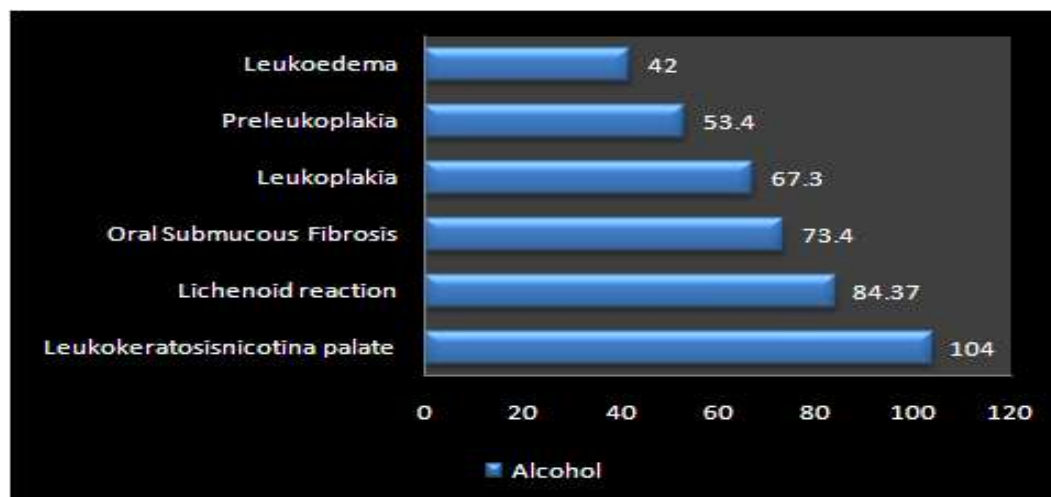n leukoplakia – 127.3, leukoedema – 108.7, OSMF 18.45, preleukoplakia – 16, lichenoid reaction – 12 (Graph 8b) non-smokers. Similar trends were seen among rural smokers[10].

The prevalence of tobacco consumption in our study was 24% (778/2509); out of which 87% (676) were males and 13% (101) were females. Even though the prevalence was less in females, they have a more risk of malignant transformation[11]

A study conducted by Neufeld and his coworkers[12] provides national estimates of regular tobacco and alcohol use in India and its association with gender, age and economic group, obtained from a representative survey of 471,143 people over the age of 10 years in 1995-96, the National Sample Survey. The national prevalence of regular use of smoked tobacco is estimated to be 16.2%, chewing tobacco 14.0% and alcohol 4.5%. Men were 25.5 times more likely than women to report regular smoking, 3.7 times more likely to regularly chew tobacco, and 9.7 times more likely to regularly use alcohol.

Rani M et al[13] in 2003 in a cross sectional, nationally representative population based household survey concluded that in 30 % of the population, 15 years or older-47% men and 14% of women-either smoked or chewed tobacco.

Smoking, drinking and chewing have been positively associated with oral lesions such as oral submucous fibrosis (OSF), leukoplakia, lichenoid reaction, smoker's palate which have the potential of malignant transformation.

In our study 218 cases were found positive for OPMD i.e. 8.6% from total population and 28.02% from habit positive cases.

Saraswathi TR et al[14]in 2006 in a hospital based study from South India found that oral soft tissue lesions were found in 4.1% of the study subjects. Prashant B. Patil et al[15]in 2013 in a hospital based cross-sectional study in South India found that oral mucosal lesions were present in 322 (26.8%) subjects who had tobacco smoking and chewing habits.

In a study conducted in Indore, Madhya Pradesh by Priyanka Mahawaret al[16] in 2011, chronic tobacco chewers were screened for oral pre-malignant lesions followed by an educational intervention about the harmful effects of tobacco and found that among 80 identified chronic tobacco chewers, 60 were males and 20 were females. Lesions such as leukoplakia, erythroplakia and oral sub-mucosal fibrosis were found in 10 females (50%) and 24 males (40%).

In our study, the prevalence percentage of each of the potentially malignant disorder from total screened population was preleukoplakia (0.2%)   lichenoid reaction (0.3%), leukoplakia (1.3%), leukoedema (1.5%), leukokeratosisnicotina palate (1.8%), Oral Submucous Fibrosis (4.74%)

In a hospital based study from Vidisha(60 kms from Bhopal), Ravi Mehrotra et al 2010[17] found that with reference to the habit of tobacco use, 635(21%) were smokers, 1272(42%) tobacco chewers, 341(11%) smokers and chewers, while 1464(48%) neither smoked nor chewed tobacco. 256 patients were found to have significant mucosal lesions. Of these, 216 cases agreed to undergo scalpel biopsy confirmation. 88 had leukoplakia, 21 had oral submucous fibrosis, 9 showed smoker's melanosis and 6 patients had lichen planus, while there was 1 patient with lichenoid reaction.

In a South Indian study by Saraswathi TR et al in 2006[14] oral soft tissue lesions were found in 4.1% of the study subjects. In this study, smoker's melanosis was found to be the most common soft tissue lesion with the prevalence being 1.14%. Stomatitis nicotinapalatini (0.89%) and leukoplakia (0.59%) were the second and third most common lesions. Among men, smoker's melanosis and stomatitis nicotinapalatini were more prevalent as compared to other soft tissue lesions, whereas among women leukoplakia and OSMF were more prevalent.

In our study the overall prevalence of leukoplakia was 1.8%from total population and 4.8% among tobacco users. It was less than that reported in a number of epidemiologic studies, like 8.25% reported by Prashant B. Patilet al[15] and 11.5% reported by Bhowate et al[18].

Campisi and Margiottaet al[19,] in their Italian study reported one of the highest incidences (13.8%) of leukoplakia in men who drank alcohol and had the habit of smoking.

Lichenoid reactions had a prevalence of 2.9% among tobacco users and 0.3% among total sample, which is higher as compared to the study by Prashant B. Patil et al 2013[15].

Lichenoid reaction was more prevalent among the cases having combination of tobacco chewing and alcohol. In 2018, in a study in Mahabubnagar, Telengna, authors reviewed a totalnumberof 1453 oral biopsies and found that 62cases were potentially malignant disorders with themost common lesion being luekoplakia[20]. Leukoplakia is more common in males and is6 times more prevalent among smokers[21].

CONCLUSIONS:

The multi-center cross-sectional study revealed that the deleterious habits in males showed a prevalence of 87% and in females showed a prevalence of 13%. The ratio of male: female prevalence was determined to be 7:1. The commonest oral lesions were determined as per their levels of prevalence as OSMF (4.74%) >Leukokeratosisni- cotina palate (1.8%) >leukoedema (1.5%).

The least common in our series was pre-leukoplakia (0.20%). The chewing index showed a POSD of 2.8% for T – value 0.035 indicating it was statistically not significant in OSMF and Leukoplakia statistic. When the chew index was analyzed between leuko-keratosisnicotina palate and OSMF which was highly significant with a T – value 4.48 for POSD of 100%. The smoking index was found to be a sensitive indicator and for a T – value of 0.532 a moderate POSD of 40.34% emerged. The leukokeratosisni-cotina palate and lichenoid reaction when compared gave a T – value of 5.569 pointing towards 100% difference in POSD. The alcohol index was a definitive discriminator between leukokerat-osisnicotina palate andlichenoid reaction. The T – value being 1.018 and POSD indicating 68.61% which was statistically significant. The leukokerat-osisnicotina palate and leukoedema as expected showed a POSD of 100% for a T – value of 6.982.

The prevalence rates of oral lesions show a logically observational relationship to the habit index of chewing, smoking, and alcohol. The role of oral physician in temperance of oral habits and in forcing government policy decisions to protect our youth and school children from bad habits on oral mucosa and general health appears to be a goal which is unsurmountable due to commercial advertising and tax compulsions of the state and central government.

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Antimicrobial and Antibiofilm Activity of L-Arginase Produced by Streptomyces sp. HAB 228

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ABSTRACT

Several antibiotics are presently available in the market that are reported to be active against opportunistic pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans*. On entering host immune system these infectious bacteria are difficult to treat due to pre-existing drug resistance mechanisms and also through their biofilm forming abilities. Thus, demanding alternative therapeutic strategies for targeting drug resistant and biofilm forming bacteria. The purified L-arginase inhibited the growth of *Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans* under *in-vitro* conditions. Antimicrobial and antibiofilm forming ability of L-arginase enzyme produced by *Streptomyces* sp. HAB 228 is noted. It is also observed that the reduction in biofilm formation being 57±4%, 54±6% and 29±4% of *Pseudomonas aeruginosa, Streptococcus mutans* and *Staphylococcus aureus* respectively by 25 U of L arginase.

KEY WORDS: antimicrobial, antibiofilm, biofilm, L- arginase, *Streptomyces* sp.

INTRODUCTION:

Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans are generally linked with both acute and chronic infections of humans and animals like dental diseases, ear infections, eye infections, urinary tract infections, implant rejections etc.[1] They generally resist antibiotictreatment due to various reasons predominantly including their ability to form biofilms.

Biofilm is a complex biological system comprising of extracellular matrix (exopolysaccha-ride) of microorganisms along with proteins, lipids, nucleic acidsis dynamic and is comparativelyless defined micro-habitat[2]. Biofilms formed by the pathogenic microorganisms are detrimental not only for human health but also for industries since they cause blockade and corrosion of filters, pipelines, storage tanks etc[3].

L-arginase, an enzyme that hydrolyses L-arginine into ornithine and urea is gaining importance now-a-days due to its anticancer effects.[4] Various microorganisms like *Cyanobacteria, Bacillus anthracis, Arthrobacter* sp. KUJ 8602, *Agaricusbisporus* etc, have been reported to produce L-arginase. L- arginine is considered as one of the most important precursorand intermediate in metabolic cycle[5, 6, 7]. Hence, limiting its supply by L-arginase supplementation may lead to inhibition in growth of microorganisms and/ or formation of biofilm by microorganisms. Thus the present study was conducted to assess antimicrobial and antibiofilm effects of L-arginase produced by *Streptomyces* sp.

MATERIALAND METHODS:

L-arginase production and purification: The production of L-arginase was done on Czapek Dox Soil Extract Arginine(CDSEA) broth containing (gm/L), Sodium nitrate- 2.0 gm, Di potassium hydrogen phosphate- 1.0 gm, Magnesium sulphate-0.5 gm, Potassium chloride-0.5 gm, Ferrous sulphate-0.01 gm, Soluble starch-30.0 gm, L-arginine- 0.15 gm, Soil extract- 50.0 ml for detection of extracellular production of arginase. After inoculation the flask was incubated at 28±2°C for 8 days in BOD incubators. Quantification of extracellular arginase produced by *Streptomyces* sp. was done in cell free culture broth obtained by centrifugation of culture broth at 5000 rpm for 10 minutes at 4°C followed by filtration of

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supernatant through Millipore syringe filters (0.22μm).

L-arginase activity: An aliquot of 0.8 ml of buffered arginine was mixed with 1.0 ml of enzyme and incubated at 37 °C for 30 minute in water bath. On completion of incubation period, 0.2ml of TCA (10% w/v) solution was added to stop the reaction and kept for 10 minutes followed by centrifugation at 10,000 rpm for 10 minutes at 4°C. To 1 ml of supernatant 3ml of chromogenic solution was added and boiled for 15 minutes at 100°C. The absorbance was read at 525 nm using Picodrop spectrophotometer. Reaction blank was processed as above, with addition of TCA before incubation. One Unit (U) of L Arginase activity is considered here as 1μmole of urea produced per minute at 37°C.

Purification of L-arginase: Purification of L-arginase was done using ammonium sulphate salting out method, complete precipitation of protein was achieved at 75 % saturation. Followed by dialysis of precipitated crude enzyme was done using 10 KD a cut-off dialysis membrane in 0.001 M PBS, overnight. Separation and purification of L-arginase was done using Sepahdex G-100 column and eluted using Tris-HCL buffer, pH 8.0. The steps of purification were performed in sequential manner described above.

Anti-microbial assay of L-arginase using agar well assay: Antimicrobial activity of L-arginase against *Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans* was studied using agar well diffusion technique. Agar well was made on Blood Agar and Brain Heart Infusion Agar media plates using sterilized cork borer and 50 μL of L-arginase (20U) was dispensed in each well. Thereafter, the spore suspension of bacteria prepared in Tween 20 - water blanks (100:1v/v) of density equivalent to 0.5 OD at 700nm was spread uniformly on the surface of media plates. The inoculated plated were incubated at 37⁰C for 24 hours. Subsequently, plates were observed for formation of zone of inhibition in growth of micro-organisms.

Biofilm inhibition assay: Quantitative estimation of anti-biofilm potential of L-arginase was studied 96 well, flat bottom micro-titre plates. 100 μL of Brain heart infusion broth containing 2% (w/v) was dispensed in each well and was subsequently inoculated with 50 μL of bacterial spore suspension. 32 well were kept as control to which L-arginase was not added and the volume was made up using PBS (pH 7.2), 50μL of L-arginase was added in each well in increasing concentration (1U, 5U, 10 U, 15 U, 20 U, 25 U). Incubation was done at 37°C for 24 hour, thereby

the supernatant was removed from each well and washed thrice with sterilized PBS. The biofilms were stained with 150μl of Gram's Crystal Violet for 1-2 minutes. The excess stain was removed by washing. The biofilm formed was estimated by dissolving it using 30% (v/v) glacial acetic acid. The elute from each well was centrifuged at 5000 rpm for 10 min to remove debris. The absorbance of dissolved Gram's Crystal Violet dye was read at 630 nm. The percentage of biofilm formed was calculated using equation[8]

Biofilm formation (%)= (Optical density in presence of L-arginase/ Optical density of blank)x 100

RESULTS:

Extracellular production of L arginase by *Streptomyces* sp. HAB 228 on CDSEA broth had yield of 0.28 ± 0.007gm growth (dry weight). The cell free culture broth displayed 137.8 ± 2.8 U/ml of L-arginase activity. On purification of enzyme the purity reached 82.3 % with recovery of 57% enzyme.

L-arginase produced by *Streptomyces* sp. HAB 228 was found to inhibit the growth of all herein tested pathogenic bacteria, *Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans,* under *in vitro* conditions (Graph 1).The extent of inhibition noticed on different growth media differed as thezone of inhibition of *Pseudomonas aeruginosa* in Brain Heart Infusion Agar was 25±3 mm (Figure 1), while it was 9±2 mm on Blood agar. Similarly the zone of inhibition with *Staphylococcus aureus* and *Streptococcus mutans* was found more on BHI media as compared to blood agar.

Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans were found to form biofilms on microtitre plates when studied under Stereo-microscope (Figure 2). The inhibition in formation of biofilm was noticed on addition of L-arginase along with bacterial culture *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus mutans*. The biofilm formation was measurably reduced in case of *Pseudomonas aeruginosa* followed by *Streptococcus mutans* (Table 1).

DISCUSSION:

Biofilm contains gradation of nutrients and oxygen on moving from exterior to interior. Hence, bacteria located in nutrient deprived area have reduced metabolic activity as well as slow growthrate.[9] These bacteria can thus be considered as dormant andhence may render resistance and tolerance to antibiotics.

Bacteria in different layers of biofilm communicate with each other through various

Graph1: Inhibition in growth of pathogenic bacteria by L- arginase produced by *Streptomyces* sp. HAB 228 using Agar well diffusion technique on solid media under *in vitro* conditions

Table 1: Effect of purified L- arginase on biofilm produced by *Pseudomonas aeruginosa, Streptococcus mutans* and *Staphylococcus aureus*.

L- arginase (U*)	Inhibition in Biofilm Formation (%)		
	Pseudomonas aeruginosa	*Streptococcus mutans*	*Staphylococcus aureus*
1	-	2±1	-
5	16±4	6±2	-
10	23±9	11±5	2±0
15	34±7	19±4	7±0
20	53±5	48±7	18±3
25	57±4	54±6	29±4

One Unit (U) of L-arginase activity is considered here as 1 μmole of urea produced per minute at 37˚C.

Figure 1: Zone of inhibition of growth *Pseudomonas aeruginosa* in Brain Heart Infusion Agar, incubated for 24 hours at 37˚C well diameter 8 mm.

biomolecules. Removal of such molecules from the vicinity of bacteria may be promising for controlling biofilm formation. Other approaches may comprise using enzymes able to degrade biofilm matrix chiefly composed of extracellular DNA, protein, exopolysaccharides and dead cells.[10]L-arginase was analysed for its inhibitory effect on both planktonic cells and biofilm.

Herein tested bacteria *Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans* are amongst commonest pathogens related to nosocomial infections.[11] Treatment of staphylococcal infections poses challenges due to its resistance to antibiotics, multiplicity of virulence factors and its ability to form a biofilm.[12,13]The growth of microorganisms may be accentuated by Blood agar, the mediumenriched with high protein and essential growth factors.[14] Although it is evidenced herein in
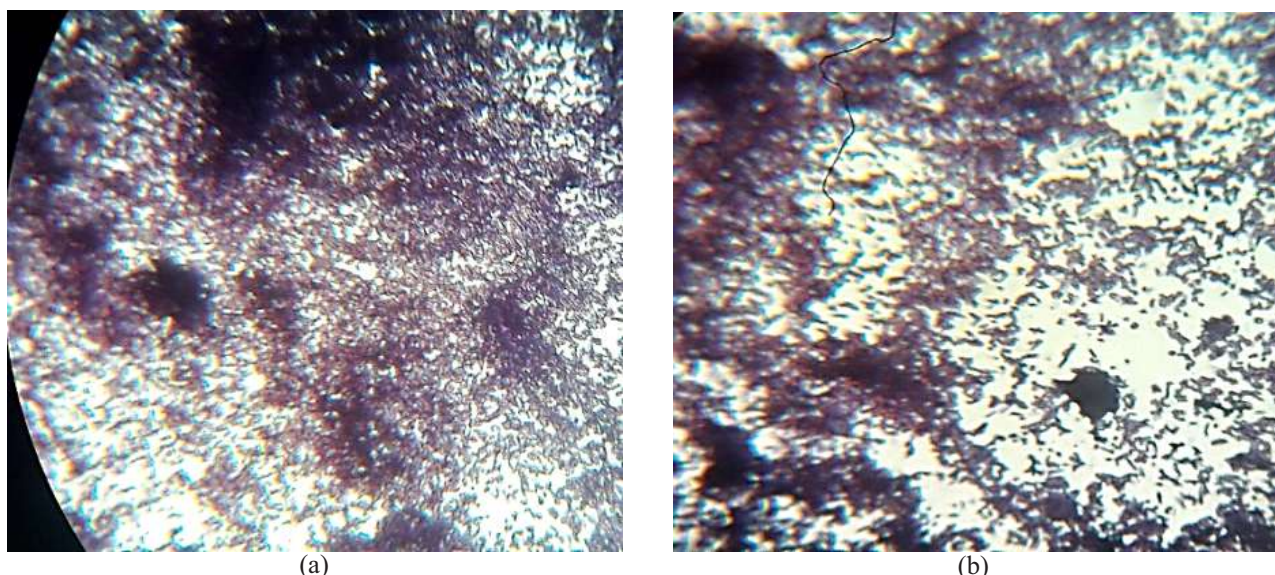
Figure 2: Biofilm formed by *Streptococcus mutans* on micro titre plate grown in BHI medium (a) Control: Not treated with L-arginase; (b) Treated with 25 U of L-arginase.

this research that L-arginase has proven role in minimizing the arginine availability but it may be compromised due to additional sources of its availability and hence there is noted lesser inhibition in blood agar as compared to BHI media.

Microbial biofilms have multidimensional structures covered with viscous polymeric milieu with different phenotype, genetic composition and functional expression as protein synthesis in comparison to its planktonic form.[15,16]

Bacterial proteins such as fibronectin-binding protein A (FnBPA) help bacteria to resist mechanical disruption further blocking diffusion of natural and artificial antimicrobial agents.[17, 18] Our study reported activity of L-arginase against planktonic stage along with inhibition of biofilms produced by *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus mutans* at different concentration although extent of inhibition increased with enzyme units initially which got stabilized at higher concentration.

CONCLUSION:

It is concluded that the novel strategies for treatment, as have been adopted and are usually naturally preferable, should be using enzymes in infective cases as these may be better tolerated, acceptable and user friendly than the existing antibiotics due to their inherent known limitations. However, while underlining the need for further necessary studies, it is also inferred that the pharmacologically suited, disease specific and system

efficient strategies for synergistically active combined treatments i.e. using enzymes along with the existing norms may also be considered especially for those ailments associated with blood abnormalities. These recommendations need support of further studies prior to field level testing.

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Hypocalcaemia Assessment: An Efficient Measure for Prevention of Seizures

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ABSTRACT

The present study aimed to assess and compare the levels of calcium in cases and controls with and without seizure disorders respectively. It also aimed to assess association of serum calcium levels with time since seizures and causes of seizures. This case control study conducted in Department of Medicine, People's Hospital Bhopal for a period of 18 months included 77 cases with seizure disorders and equal number of controls without seizure disorders. Data regarding detailed history regarding presenting complaints, time since onset of seizures, causes of seizures was obtained from cases. Mean calcium level amongst cases was 8.99±0.75 mg/dl and that amongst controls was 9.32±1.11 mg/dl. The present study observed significantly lower calcium level amongst cases as compared to controls ($p<0.05$). No statistically significant association of mean calcium level was observed with time since seizures and various causes of seizures ($p>0.05$). Serum calcium levels has significant association with seizures since there is some level of hypocalcemia in few though not all seizure patients. Calcium supplementation may be considered in patients with intractable seizures and in patients with drug refractory seizures. Thus calcium levels must be evaluated in every seizure patient irrespective of cause.

KEY WORDS: hypocalcemia, seizure, serum calcium

INTRODUCTION:

The word seizure is derived from a Latin word sacire meaning 'to take possession of". Seizure is a convulsive incident due to aberrant, excessive, hypersynchronus emissions from an aggregate of CNS neurons.[1] The incidence rate of seizures varies from 38 to 49.3 per 1 lakh population per year in India.[2] When burst of electrical impulses in brain exceed the normal limits, it leads to seizure. Once generated, these impulses then spread to the adjacent neurons and results in uncontrolled electrical activity in brain. These may manifest as tonic clonic seizures characterized by uncontrolled jerking movements or may present as absent seizures i.e. momentary loss of awareness.[3] Neuronal excitability is controlled by the balance between excitatory an inhibitory effects, both intrinsic and synaptic.[4]

The etiology of seizures vary with age, e.g. trauma, cerebrovascular accidents, brain tumors and metabolic causes are common cause of seizures in older age groups.[5] Hypocalcemia-induced seizures are also one of the common cause of seizures. These seizures usually occur in patients with preexisting endocrinological abnormalities associated with poor calcium homeostasis.[6] The role of calcium in seizure disorders could be attributed to reduced excitatory threshold, increased neural transmission, and increased neuromuscular excitation due to hypocalcemia.[6] Also hypocalcemia can also be responsible for raised intracranial pressure, impaired cerebral functions and high susceptibility of neurons of hippocampus for epilepsy.[7] The present study aimed to assess and compare the levels of calcium in cases and controls with and without seizure disorders respectively. It also aimed to assess association of serum calcium levels with time since seizures and causes of seizures.

MATERIALS AND METHODS:

The present case control study was conducted in Department of Medicine, People's Hospital Bhopal during 1st December 2017 to 30th May 2019. The study included 77 cases with seizure disorders and equal number of controls without seizure disorders. Ethical clearance was obtained from institute's ethical committee. All the participants were explained the

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Table 1: Distribution of socio-demographic variables among cases and controls.

Sociodemographic variables		Cases (n=77)		Controls (n= 77)		p value
		Frequency	Percentage	Frequency	Percentage	
Age group	=20	10	13.0	6	7.8	
	21-30	8	10.4	7	9.1	
	31-40	9	11.7	19	24.7	0.29
	41-50	15	17.5	18	23.4	
	51-60	14	18.2	9	11.7	
	>60	21	27.3	18	23.4	
Gender	Male	46	59.7	57	74.0	
	Female	31	40.3	20	26.0	0.06

nature and purpose of study, and they were ensured that confidentiality would be maintained. The inclusion criteria for cases comprised of (a) patients diagnosed as having seizures admitted in People's Hospital and, (b) age group of 15 to 70 years of either gender. Those excluded were (a) patients not giving consent to participate in the study, (b) patients presenting after 72 hours from the onset of seizure, (c) pregnant and lactating females and/or (d) taking oral/parenteral magnesium and calcium. Similarly the inclusion criteria for participants of control group comprised of age and gender matched individuals without history of any seizure disorders in the age range of 15 to 70 years. However, individual not giving consent to participate in the study, pregnant/ lactating females or taking oral/parenteral magnesium and calcium were excluded from the control group.

Those willing to participate and fulfilling the inclusion criteria of either group were enrolled during the study period. The option to withdraw from the study was always open. Details regarding sociodemographic variables was obtained from all the study participants of either group and entered in questionnaire. Data regarding detailed history regarding presenting complaints, time since onset of seizures, causes of seizures was obtained from cases. Blood sample was obtained from all the participants under aseptic precautions and subjected to RBS and Serum calcium estimation.

Data was compiled using MS excel and analyses using SPSS software version 20. Frequency was calculated for grouped data and expressed as percentage whereas numerical data was expressed as Mean and SD. Chi square test was used to assess the difference between quantitative variables whereas mean values between the groups were compared using independent t test. Analysis of variance (ANOVA) was used to compare means between 3 variables. p value less than 0.05 was considered significant, whereas p value less than 0.01 was considered highly significant.

RESULTS:

The present study included 77 cases with mean age of 44.74 ± 16.73 years and 77 controls with mean age of 45.44 ± 15.52 years. Majority of cases in present study belonged to more than 60 years of age (27.3%), whereas majority of patients in control group belonged to 31 to 40 years of age. Maximum patients in both the groups were males i.e. 59.7% amongst cases and 74% amongst controls. However test of significance observed no statistical difference in age and gender composition between cases and control ($p > 0.05$) (Table 1).

Mean RBS in cases and controls was 129.64 ± 47.35 and 128.84 ± 31.79 mg/dl respectively in present study. Test of significance (unpaired t test) observed no statistical difference in mean RBS between cases and controls ($p > 0.05$). Mean calcium level amongst cases was 8.99 ± 0.75 mg/dl and that amongst controls was 9.32 ± 1.11 mg/dl. The present study observed significantly (unpaired t test) lower calcium level amongst cases as compared to controls ($p < 0.05$) (Figure 1).

In present study, mean calcium level within 24 hours of seizures was 8.94 ± 0.72 mg/dl whereas as mean calcium level between 24-48 hours and >48 hours was 9.15 ± 0.88 and 8.88 ± 0.64 mg/dl respectively. Test of significance (ANOVA) observed no statistically significant difference in mean calcium level amongst cases with time since seizures ($p > 0.05$). (Table 2) The present study observed no statistical difference in mean calcium level in various causes of seizures ($p > 0.05$) (Table 3).

DISCUSSIONS:

A seizure can be described as a paroxysmal event which can be due to abnormal, unnecessary, hypersynchronus discharges from an collection of CNS neurons.[1] The present study assessed the serum calcium levels in case with seizure disorders and
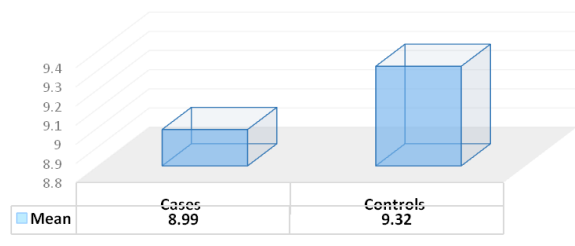
Table 2: Association of time since seizure with calcium level amongst cases.

Calcium (mg/dl)	Time since seizure (Hours)		
	<24	24-48	>48
Mean	8.94	9.15	8.88
SD	0.72	0.88	0.64
p value	0.53		

Table 3: Association of cause of seizure with magnesium level amongst cases.

Calcium (mg/dl)	Cause		
	Organic	Metabolic	Unspecified
Mean	8.96	8.84	9.50
SD	0.73	0.69	0.93
p value	0.105		

compared them with healthy controls. Serum calcium levels are often associated with serious complications such as arrhythmias, seizures etc. Hypocalcemia has been defined asserum calcium level of <8.5mg/dl or ionized calcium level of <4 mg/dl.[8] The normal serum calcium levels are maintained within very narrow range for optimal extracellular and intracellular functions.[9] Normal level of serum calcium is maintained by multiple factors such as parathyroid hormone (PTH), calcitriol, vitamin D, serum magnesium serum phosphate level and ionized Ca itself.[10] Thus alterations in Vitamin D, magnesium, PTH and calcitriol level may present with altered calcium levels.

Figure 1: Calcium level (mg/dl) among cases and controls.

The present study observed significantly (unpaired t test) lower calcium level amongst cases i.e. 8.99 ± 0.75 mg/dl as compared to controls i.e. 9.32 ± 1.11 mg/dl ($p<0.05$).These findings are in concordance with the findings of Abdullahi I et al where mean serum calcium in the 60 cases was significantly lower than the control cases (2.3 ± 0.13 mmol/L vs. 2.4 ± 0.12 mmol/L; $p < 0.001$).[11]

However, the reference study was conducted to assess the association of magnesium levels with seizure disorders and hypocalcemia might be secondary to magnesium imbalance. Victor et al in their study concluded that acute hypocalcemia is associated with increased neuromuscular excitability and tetany and thus the patients may present with seizures and altered mental status.[12]

Calcium supplements in seizure disorders are known to inhibit ssmall conducting Na^+ leak channel (NALCNs) and shifts dependency of voltage-gated sodium channels, reduces inward current through AMPA channels, and depresses the release of excitatory neurotransmitters and thus are helpful in reduction of seizure activity. This might explain the possibility of hypocalcemia in cases of seizures.[13]

To our knowledge none of study have assessed the association of calcium levels with time since onset of seizures and etiologies. However the present study observed no statistically significant association of serum calcium levels with time since seizures as well as various causes.

CONCLUSION:

Serum calcium level has significant association with seizures since there is some level of hypocalcemia in few though not in all seizure patients. Calcium supplementation may be considered in patients with intractable seizures and in patients with drug refractory seizures. Thus calcium levels must be evaluated in every seizure patient irrespective of cause since it may help in preventing seizures.

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Clinico-radiological Presentation of Pulmonary Tuberculosis in Diabetes Mellitus

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ABSTRACT

An observational study was conducted to determine various clinical and radiological presentations of PTB in diabetes mellitus. All patients with PTB and Diabetes mellitus, newly detected(PND) or known diabetes(PKD),after a detailed history and thorough examination, were subjected to sputum smear examination for AFB, FBS, PPBS, HbA1c and Chest X-rays. Out of 78 patients with Pulmonary Tuberculosis and Diabetes mellitus,46 (59.0%) patients were PND and 32 (41.0%) were PKD. There was male preponderance in both groups.28% of the PKD patients had diabetes for 1-2 years. In the PND group, 31(67.4%) patients were sputum smear positive,15 (32.6%) were sputum smear negative. Forty six (100%) had cough with sputum and weight loss.Radiologically, 36(78.3%) patients had lesions in lower lung field, 18 (39%) had minimal lesions , 33(71.7%) had right sided lesions,9 (19.6%) patients had multiple cavities and 31(67.4%) patients did not have cavities. In the PKD group, Majority (69.6%) of the patients had poor glycemic control with HbA1C>8,23(72%) patients were sputum smear positive ,9(28%) patients were sputum smear negative,32(100%) patients had cough with sputum and weight loss.Radiologically,25(78%) patients had lesions in upper lung field and had right sided lesions.,12(37.5%) patients had multiple cavities and 16(50%) did not have cavities. Higher incidence of cavities was seen in the PKD group.

Patients with PTB and Diabetes were mostly male in both groups. Cough ,sputum and weight loss were the most frequent symptoms in both PKD and PND. Fever was less common in both the groups. Both PND and PKD were more likely to be sputum positive(67.4% vs 72%) than sputum negative(32.6% vs 28%). Radiologically, there was a higher involvement of upper lung field(78%) in PKD group as compared with PND group and extent of lesion was almost similar in both the groups. Location of cavitary lesion over upper lung field(34.6% vs 6.4%) and multiple cavities (37.5% vs 19.6%) were more in PKD group as compared to PND group.

KEY WORDS: cavities, clinico-radiological, diabetes mellitus, lesion, pulmonary tuberculosis

INTRODUCTION:

Tuberculosis, which affects millions of people each year, is a major global health problem. It is still the main reason for mortality and morbidity in developing countries[1]. The most common Diabetes mellitus globally is Type 2 Diabetes Mellitus (T2DM) [2]. T2DM is recognized as a major risk factor for the progression of active Pulmonary Tuberculosis (PTB), although the mechanistic link between DM and Pulmonary Tuberculosis remains poorly understood[3]. DM has been documented as a risk

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factor for Tuberculosis for decades. According to the International Diabetes Federation 2013[4], 382 million people were living with DM, of whom 80% were in low and middle-income countries and This is estimated to increase to 592 million by 2035. There were 65.1 million cases of DM in India that is estimated to rise upto 109 million by 2035 [4].

Higher prevalence of Pulmonary Tuberculosis in patients of DM is a well- known fact for a long time. However, higher prevalence of impaired glucose tolerance and DM in a tuberculous population is also being increasingly realized now and it becomes more relevant due to increased prevalence of DM in general population. Since, both Diabetes mellitus and Tuberculosis are major public health problems, we carried out the present study to determine various clinical and radiological presentations of Pulmonary Tuberculosis in patients with Diabetes mellitus in Central India. Specifically, we studied the differences in the clinico-radiological presentations between

Pulmonary Tuberculosis in newly detected Diabetes mellitus(PND) and Pulmonary Tuberculosis with known Diabetes mellitus (PKD).

MATERIALS AND METHODS:

This was an observational study. All patients of Pulmonary Tuberculosis with DM who came to Pulmonary Medicine OPD of People's College of Medical Sciences and Research Centre, Bhanpur (MP) were enrolled. The study was conducted from January 2018 to June 2019. Group 1 comprised patients with Pulmonary Tuberculosis with newly detected DM type-2 (PND) and Group 2 had patients with Pulmonary Tuberculosis with known DM type-2 (PKD).

The Inclusion criteria were: Patients with age more than 18 years of either gender; Patients with a confirmed diagnosis of Pulmonary Tuberculosis; Patients with known DM type-2 on glucose-lowering medications; Patients with newly detected DM 2 and Patients and/or his/her legally acceptable representative willing to provide voluntary written informed consent to participate in the study.

The Exclusion Criteria were: Patients with age less than 18 years; Patients living with HIV and AIDS; Patients with any type of malignancy, Pregnant women; Patients with extrapulmonary tuberculosis; Patients with DM type-1 and Patients and/or his/her legally acceptable representative not willing to provide their voluntary written informed consent to participate in the study.

All presumed TB patients who had any one of the following: cough>2weeks, fever>2weeks, significant weight loss, hemoptysis, any abnormality in chest X- ray were asked to submit first sputum sample (spot sample) and next morning sputum sample for AFB. Diagnosis of Pulmonary Tuberculosis was made by sputum smear examination for AFB by Ziehl-Neelsen technique and in sputum smear negative patients, diagnosis was made by molecular test i.e. Cartridge Based Nucleic Acid Amplification Test (CBNAAT).

DM status was assessed after reviewing the available records with the patients and/ or their self-reported history regarding DM status. All patients with newly diagnosed Pulmonary Tuberculosis underwent FBS, PPBS and HbA1c as a part of screening and diagnosis of Diabetes was made according to 'Standards of Medical Care in Diabetes 2017'.

Radiological evaluation with plain CXR was done to note the location of lesions, the extent of lesions, location of cavity and number of cavities.

Extent of disease was classified as follows:
1) Minimally advanced: lesion which was slight to moderate in density with no demonstrable cavitation, the total volume of lung on one side ,present above the second chondrosternal junction, and spine of the fourth thoracic vertebra.
2) Moderately advanced: Disseminated lesions of slight to moderate density that extended to the total volume of one lung or equivalent in both lungs ; and a total diameter of cavitations, if present, less than 4 cm.
3) Far advanced: lesion more extensive than moderately advanced.

Both right and left lung parenchyma were divided into upper and lower lung fields by a horizontal line drawn across the hilum.

The data was entered into Microsoft Excel for analysis. Online statistical software was used for calculating p values. Intra group mean comparisons were done using paired 't' test. Association between two non-parametric variables was done using the Pearson Chi-square test.

RESULTS:

Out of 78 patients with Pulmonary Tuberculosis and DM, 46 (59.0%) patients were in PND group and 32 (41.0%) were in PKD group.

The mean age in PND group was 49.67 ± 12.17 years and in PKD group was 53.41 ± 9.22 years. The difference was found to be statistically not significant (p=0.147).There were 15 (32.6%) females and 31 (67.4%) males in the PND group. In the PKD group, there were 7 (21.9%) females and 25 (78.1%) males. There was a male preponderance in both the groups. TB with diabetes was present in 32 alcoholics (41%) and 45 smokers (57.7%). Smoking (62.5% vs 54.3%) and alcoholism (50% vs 34.8%) were more present in the PKD group as compared to PND group. In PKD group, there were 5 (15.6%) patients with duration of diabetes of <=1 year, 9 (28.1%) patients had 1-2 years, 5 (15.6%) patients for 2-3 years, 7 (21.9%) patients for 4-5 years, 4 (12.5%) patients for 6-10 years and 2 (6.3%) patients for more than 10 years. Majority of the patients had duration of diabetes of 1-2 years, followed by a duration of 4-5 years (Table 1).

Majority 31 (67.4%), of the patients were sputum positive and 15 (32.6%) were sputum negative in PND (p value=0.002). In PKD, majority 23 (71.9%) of patients were sputum positive and 9 (30.8%) patients were sputum negative in old DM (p=0.001).

Table 1: Baseline Characterstics.

Age	PND	PKD	Total
<30 years	2(4.3%)	0 (0.0%)	2(2.6%)
31-40 years	10(21.7%)	2(6.3%)	12(15.4%)
41-50 years	15(32.6%)	11(34.4%)	26(33.3%)
51-60 years	9(19.6%)	12(37.5%)	21(26.9%)
>60 years	10(21.7%)	7(21.9%)	17(21.8%)
Total	46(100.0%)	32(100%)	78(100%)
Mean Age	49.67 ± 12.17	53.41 ± 9.22	p = 0.147
Gender	**PND**	**PKD**	**Total**
Female	15(32.6%)	7(21.9%)	22(28.2%)
Male	31(67.4%)	25(78.1%)	56(71.8%)
Total	46(100.0%)	32(100.0%)	78(100.0%)
Personal Habits	**PND**	**PKD**	**Total**
Smoking	25(54.3%)	20(62.5%)	45(57.7%)
Alcohol	16(34.8%)	16(50.0%)	32(41.0%)
Duration of Diabetes		**Number**	**Percentage**
<=1 year		5	15.6
1-2 years		9	28.1
2-3 years		5	15.6
4-5 years		7	21.9
6-10 years		4	12.5
>10 years		2	6.3
Total		32	100.0

In both groups, most common symptoms were cough with sputum and weight loss which were present in all 78 patients (100%) followed by fever (69.2%),chest pain (29.5%), shortness of breath(25.6%), night sweats (20.5%) and haemoptysis (16.7%). The presenting complaints were similar in both the groups (Table 2).

Radiological profile of TB diabetics. Majority of the PND patients (78.3%) had lesions in lower lung field whereas in PKD group, majority of the patients (78.1%) had lesions in upper lung field. The location of lesions with regard to lung field in both the groups was distributed nearly equally. In the PND group, most (39.1%) of the patients had minimal lesion followed by moderate advanced (34.8%) and far advanced (26%) whereas in PKD group, most (40.6%) of the patients had moderately advanced lesions followed by minimal lesion (37.5%) and far advanced (22%). The extent of lesion was almost equal in both groups (Table 3).

Higher percentage of cavity (34.4%) was seen in the upper lung field in PKD group (p=0.002) and lower lung field (26.1%) in PND group. In PND group, 15 (32.6%) patients had cavities and 31 (67.4%) patients had no cavities while in the PKD group, 16 (50.0%) patients had cavities and 16 (50.0%) patients had no cavities. Higher incidence of cavities was seen in the PKD group. Multiple cavities were commonor in the PKD than in the PND group.

DISCUSSION:

This was a hospital based observational study done over a period of one and half year at the Department of Pulmonary Medicine, People's Hospital, Bhopal. Screening of all patients for Pulmonary Tuberculosis and Diabetes mellitus coming to Pulmonary Medicine OPD of People's

Table 2: Clinical profile of patients.

Sputum for AFB	PND	PKD	Total
Scanty	4(8.7%)	3(9.4%)	7(9.0%)
1+	8(17.4%)	6(18.8%)	14(17.9%)
2+	8(17.4%)	9(28.1%)	17(21.8%)
3+	11(23.9%)	5(15.6%)	16(20.5%)
Negative	15(32.6%)	9(28.1%)	24(30.8%)
Total	46(100.0%)	32(100.0%)	78(100.0%)
Sputum Positive Cases	31(67.4%)	23(71.9%)	54(69.2%)
Sputum for AFB	**PND**	**PKD**	**Total**
Negative	15(32.6%)	9(28.1%)	24(30.8%)
Sputum Positive Cases	31(67.4%)	23(71.9%)	54(69.2%)
Total	46(100.0%)	32(100.0%)	78(100.0%)
Fisher's Exact Test (Sputum-ve & Sputum+ve)	0.002	0.001	
Presenting Complains	**PND**	**PKD**	**Total**
Cough with sputum	46(100.0%)	32(100.0%)	78(100.0%)
Fever	36(78.3%)	18(56.3%)	54(69.2%)
Loss of appetite	38(82.6%)	24(75.0%)	62(79.5%)
Weight Loss	46(100.0%)	32(100.0%)	78(100.0%)
Haemoptysis	7(15.2%)	6(18.8%)	13(16.7%)
Chest pain	14(30.4%)	9(28.1%)	23(29.5%)
Shortness of breath	12(26.1%)	8(25.0%)	20(25.6%)
Night sweats	11(23.9%)	5(15.6%)	16(20.5%)

Hospital was done. The study was conducted on those patients with a confirmed diagnosis of Pulmonary Tuberculosis with DM.

Out of 78 patients with Pulmonary Tuberculosis and Diabetes mellitus,46 (59.0%) patients were PND and 32 (41.0%) were PKD. In our study, most of the TB diabetics (48.7%) were more than 50 years of age and there was a male preponderance (67.4% vs 78.1%) in both groups. Smoking (62.5% vs 54.3%) and alcoholism (50% vs 34.8%) were more in the PKD group as compared to PND group. In the PKD group, 28.% had a duration of diabetes for 1-2 years. Majority (67.4%) of the patients were sputum positive and 32.6% were sputum negative in PND group (p value=0.002). In PKD group, majority (71.9%) of the patients were sputum positive and 30.8% patients were sputum negative in PKD group (p=0.001). The predominant symptoms in both groups were cough with sputum and weight loss which were present in all 78 patients (100%) followed by fever (69.2%), chest pain (29.5%), shortness of breath (25.6%), night sweats(20.5%) and haemoptysis (16.7%).

Radiologically, PND had predominantly lower lung field (78.3%) involvement while PKD had upper lung field (78%) involvement. In PND, most (39%) of the patients had minimal lesion whereas in PKD, most (40.6%) of the patients had moderately advanced lesions. Majority of the patients in both groups did not have cavity (60.3%). Multiple cavities (37.5%) were commonor in PKD as compared to the PND (19.6%). PKD had more cavities (34.4%) in upper lung field whereas PND had more cavities in lower lung field (26.1%).

Gender: There was a male preponderance in both PND and PKD groups (67.4% vs 78.1%). Other workers in India and abroad have also reported similar male predominance. One possible explanation for this male predominance may be that in most countries young men and elderly usually have more social labour

Table 3: Radiological findings of patients.

Location of Lesion	PND	PKD	p value	
Upper lung field	27(58.7%)	25(78.1%)	0.091	
Lower lung field	36(78.3%)	24(75.0%)	0.789	
Upper+Lower lung field	17(37.0%)	17(53.1%)	0.172	
Extent of Lesion	**PND**	**PKD**	**Total**	
Minimal lesion	18(39.1%)	12(37.5%)	30(38.5%)	
Moderate advanced	16(34.8%)	13(40.6%)	29(37.2%)	
Far advanced	12(26.1%)	7(21.9%)	19(24.4%)	
Total	46(100.0%)	32(100.0%)	78(100.0%)	
Location of Cavity	**PND**	**PKD**	**p value**	
Upper lung field	3(6.5%)	11(34.4%)	0.002	
Lower lung field	12(26.1%)	9(28.1%)	1.000, NS	
Upper plus Lower field	45(97.8%)	30(93.8%)	0.565, NS	
Number of cavities	**PND**	**PKD**	**Total**	
Single cavity	6(13.0%)	4(12.5%)	10(12.8%)	
Multiple cavities	9(19.6%)	12(37.5%)	21(26.9%)	
No cavities	31(67.4%)	16(50.0%)	47(60.3%)	
Total	46(100.0%)	32(100.0%)	78(100.0%)	
Total cavities present	15(32.6%)	16(50.0%)	31(39.7%)	

activities and more stress than women, thus favouring the transmission of tuberculosis.

Duration of Diabetes: In our study, 41% patients had previously diagnosed DM. In the previously diagnosed DM, most of the them (28.1%) had a duration of diabetes of 1-2 years followed by 22% patients who had diabetes for 4-5 years.

Results of sputum for AFB: Majority(67.4%) of the patients were sputum positive and 32.6% were sputum negative in PND group (p=0.002).In PKD, majority (71.9%) of patients were sputum positive and 30.8% patients were sputum negative (p=0.001). Most of the patients (21.5%) in both groups had grade 2 sputum positivity.

In our study, sputum positivity was equal in both PND and PKD group (67.4% vs 72%). The reason why large proportion of patients were sputum positive in PKD group was that they had more cavitary type Pulmonary TB (37.5% vs 19.6%) and more advanced lesions (40.6% vs 26.1%).

Singla et al (2006)[6] and (Dooley et al 2009)[7] in their studies found that diabetes was an independent risk factor associated with more numerous AFB on sputum smear.

Presenting Complaints: In our study, most common symptoms in TB Diabetics were cough with sputum and weight loss which were present in all 78 patients (100%) followed by fever (69.2%),chest pain (29.5%) shortness of breath (25.6%),night sweats (20.5%)and haemoptysis (16.7%). The presenting complaints were similar in both the groups. Reider HL, et al (1999)[8], Crofton J et al (2009)[9] and Girardi E et al (2017)[10] in their studies reported that cough was the most common symptom of respiratory disease, including TB, and haemoptysis appeared in about 20% of TB patients.

Jovana M Pavlovic et al (2018)[11] in their study showed that cough stayed as a dominant symptom of pulmonary TB in patients with DM.

Radiological findings:

Location of lesions according to lung field: As for roentgenographic abnormality, in our study, the location of lesions was almost same in both PND and PKD groups However, there was a higher involvement of lower lung field (78.3%) in PND; and upper lung field (78.1%) in PKD group.

Extent of lesion: In our study PND in, most (39%) of the patients had minimal lesion whereas in PKD, most (40.6%) of the patients had moderately advanced lesion. The extent of lesion was almost equal in both groups.

Ezung et al (2002)[12] in their study found that out of the 27 patients of PTB with Diabeties, 11 (40.7%) had minimal lesions, 7 (25.9%) had moderate lesions and 9 (33.3%) patients had far advanced lesions.

Cavitary lesion: Majority of the patients in both groups did not have cavity (60.3%).Multiple cavities (37.5%) were commonor in PKD group as compared to the PND (19.6%). AB Shrivastava et al(2016)[13] in their study found a higher prevalence of cavitary lung disease in TB with Diabetes (83%) as compared to PTB patients without diabetes.

Location of cavity: PKD group had more cavities (34.4%) in upper lung field whereas PND group had more cavities in lower lung field (26.1%).

Bashar et al in their study found predominant upper lung field involvement but contrary to this ,Morris et al and Bacakoglu et al found predominant lower lung field involvement.

Chiang et al (2014)[17] in their study found that proportions of DM patients with cavitary lesions over upper lung field and multiple cavities were greater in patients with poor glycaemic control than good glycaemic control (HbA1c>9%).

According to Podell B K et al. (2012)[18], increased frequency of pulmonary cavitary lesions in diabetic patients with poor glycemic control could be related to reduced expression of Th 1-related cytokines.

CONCLUSION:

Patients with PTB and Diabetes were mostly male in both groups. Cough ,sputum and weight loss were the most frequent symptoms in both PKD and PND. Fever was less common in both the groups. Both PND and PKD were more likely to be sputum positive (67.4% vs 72%) than sputum negative (32.6% vs 28%).Radiologically, there was a higher involvement of upper lung field (78%) in PKD as compared with PND and extent of lesion was almost similar in both the groups. Upper lung field (34.6% vs 6.4%) and multiple cavities (37.5% vs 19.6%) were more common in PKD as compared to PND.

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Changing Paradigm in Etiology of Non Traumatic Coma Over the Last Fifteen Years

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ABSTRACT

Developing countries like India are facing double burden of diseases i.e. India is still tackling communicable disease whereas non communicable disease are on rise. The present study was conducted in two phases at a tertiary care centre, Bhopal, was conducted to assess the change in etiology of non traumatic coma over 15 years. The first phase of study was conducted from July 2001 to November 2002 on 100 patients whereas the second phase of study was done from June 2018 to October 2019 on 215 patients. A detailed history pertaining to demographic variables and detailed clinical examination along with relevant investigations were conducted and findings were noted in questionnaire. The data thus obtained during both the phases of study was compared and difference in etiology was noted. The present study observed no significant difference in age and gender composition of patients during both the phases (p>0.05) however a statistically significant difference in the etiology of NTC was noted i.e. etiology during the phase 1 was infective (33%) whereas during phase 2 etiology were predominantly due to life style related causes such as CVA (54.4%). Our study provides a detailed description of change in etiology of non-traumatic coma over 15 years. We observed that the NTC was predominantly associated with infective causes 15 years back but in present era, non-infective causes such as CVA is the leading cause of NTC. However, infective causes of NTC still persist. So it is strongly recommended that primordial and primary prevention should be the mainstay of intervention to reduce the burden of NCD on health care facility.

KEYWORDS: changing trends, non-traumatic coma (NTC), sepsis, tertiary centre

INTRODUCTION:

Coma is a serious, life-threatening medical condition that require immediate medical care and effective treatment[1]. Non traumatic coma i.e. coma without a history of a traumatic event signify underlying pathology or may be secondary to various conditions, such as severe sepsis, poisoning and hepatic encephalopathy[2]. Other causes leading to non traumatic coma include cerebrovascular accidents, drug intoxication, metabolic disturbances, post seizure states/ status epilepticus, meningitis, encephalitis, braintumour, brain abcess etc[3]. Thus, the treatment of coma depend upon the underlying causes which if not adequately managed may be fatal. To diagnose the etiology of non traumatic coma, it is important to discriminate between structural and non-structural causes with the help of computer tomography (CT) scan[4]. Causes of structural coma include cerebral infarction, intracranial hemorrhage, intracranial malignancy and central nervous system infection (e.g. encephalitis or abscess). Non-structural coma include coma as a result of poisoning, epilepsy, extracranialinfections, circulatory shock, post-anoxic, cardiac arrest,respiratory failure, metabolic problems (such as hypoglycemia, ionic and acid–base disorders, hypothermia),hepatic encephalopathy and uremic encephalopathy[2].

The etiologies of coma also depend upon geographical location. Infections were the leading cause of non traumatic coma in developing countries as compared to developed countries[5]. However Developing countries like India are facing double burden of diseases i.e. India is still tackling communicable disease whereas non communicable disease are on rise. The rise in the incidence of non communicable disease could be attributed to lifestyle changes. Thus the present study was conducted to assess the change in etiology of non traumatic coma with the rise in non communicable diseases.

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MATERIALS AND METHODS:

The present study was designed as a comparative study to assess the change in etiology of NTC over the period of 15 years. The study was conducted in two phases at a tertiary care centre, Bhopal. The first phase of study was conducted from July 2001 to November 2002 on a total of 100 patients whereas the second phase of study was done from June 2018 to October 2019 on 215 patients.

The study included all the patients presenting to Department of Medicine and diagnosed with non traumatic coma belonging to more than 12 years of age. Patients with the history of trauma, fall or RTA or patients diagnosed to have traumatic coma upon investigations and children less than 12 years of age were excluded from the study. A detailed history pertaining to sociodemographic variables such as age, gender, mode of onset of symptoms and progression of disease to arrive at the diagnosis was obtained and entered in the questionnaire. Apart from this, thorough clinical examination of all the patients was done with special emphasis on neurological examination. Relevant investigations were conducted to arrive at a definitive diagnosis. All the patients were followed up daily during the Hospital stay and their outcome was assessed.

The data thus obtained during two phases of study was thus compared to assess the etiology of NTC during both the phases. Data was compiled using Ms Excel and analysed using SPSS software version 20. Chi square test was applied to assess the difference between two phases, p-value<0.05 was considered significant whereas p-value<0.01 was considered highly significant.

RESULTS:

The study included 100 patients during first phase and 215 patients during the second phase.

The present study observed no significant difference in age and gender composition of patients during both the phases (p>0.05). Thus the study population of both the phases was comparable. Majority of patients belonged to 61 to 80 years of age i.e. 34% during phase 1 and 34.9% during phase 2. About 56% and 65.6% patients with NTC were males during phase 1 and phase 2 of study respectively.

The infective etiology was observed in 33% patients during phase 1 whereas it was observed in only 10.7% patients during phase 2 of the study. Non infective causes attributed to 67% and 89.3% cases of NTC during phase 1 and phase 2 respectively. The present study observed a statistically significant shift in etiology of NTC from infective causes during phase 1 (33%) to non infective and life style related causes during the period of phase 2 (p<0.01).

The present study documented significantly better outcome in terms of discharge of patients during second phase of study (p<0.01).

DISCUSSION:

This study depicts the changing trends in the etiology of non traumatic coma over the period of fifteen years. Various studies have assessed the etiologies and prognosis of non traumatic coma but none have shown the trends. The inclusion and exclusion criterion throughout the study period was same. Maximum patients in both the phases belonged to 61 to 80 years of age and maximum patients diagnosed with NTC were males. The demographic variables of patients of both the phases were comparable (p>0.05). Hiremath RS et al conducted a study in 2016 also documented the most common age group of patients presenting with non traumatic coma as 51 to 60 years (26%) followed by 61 to 70 years (20%).[6]However Sarin SM et al in their study in 2016 observed lower mean age i.e.47.61 years as compared to our study[7]. Male predominance in the occurrence of non traumatic coma was supported by Wong CP et al in which the authors observed non traumatic coma in 155 male and 123 female[8].Thus non traumatic coma occur in patients with advancing age and in male patients.

Our study aimed to study the shift in etiology of non traumatic coma from infective pathology to non infective pathology as evidenced by increase in incidence of non communicable diseases. Amongst infective causes, cerebral malaria were observed in 14% cases during first phase of study whereas that after 15 years were observed in none. However, pyogenic meningitis and TBM were the cause in 19% and 4.6% patients in phase 1 and phase 2 respectively. Non infective causes were responsible for 89.3% cases during phase 2 as compared to 67% in phase 1 and the observed difference in etiologies between both the phases was statistically highly significant (p<0.01).In 2001 Wong CP et al conducted the study and observed infection as the commonest cause of non-traumatic coma, accounting for 38% of cases followed by intoxication, epilepsy, and complications of congenital abnormalities[8]. However Sarin SM et al in their study on 80 patients in 2016 documented most common cause of non traumatic coma was Cerebrovascular accident (45%) followed by metabolicencephalopathy, CNS and other infections, septicaemia and poisoning[7]. The findings of our study

Table 1: Distribution according to sociodemographic variables.

Sociodemographic variables		Phase 1 (n=100)		Phase 2 (n=215)		χ^2	p-value
		Frequency	Percentage	Frequency	Percentage		
Age group (years)	≤20	7	7	13	6	2.62	0.63 (NS)
	21-40	24	24	43	20		
	41-60	33	33	72	33.5		
	61-80	34	34	75	34.9		
	>80	2	2	12	5.6		
Gender	Male	56	56	141	65.6	2.67	0.10 (NS)
	Female	44	44	74	34.4		

Table 2: Distribution according to etiology.

Etiology		Phase 1 (n=100)		Phase 2 (n=215)	
		Frequency	Percentage	Frequency	Percentage
Infective	Pyogenic meningitis	15	15	5	2.3
	TBM	4	4	5	2.3
	Cerebral malaria	14	14	0	0
	Hepatitis	0	0	1	0.5
	Sepsis	0	0	12	5.6
Non-infective	Uremia	4	4	0	0
	DKA	3	3	9	4.2
	Hepatic	12	12	33	15.3
	Poison	5	5	21	9.8
	Hypoxia	2	2	0	0
	CKD	0	0	10	4.7
	CVA	41	41	117	54.4
	GBS	0	0	1	0.5
		χ^2=8.28; p=0.004			

Table 3: Distribution according to outcome of patients.

Outcome	Phase 1 (n=100)		Phase 2 (n=215)	
	Frequency	Percentage	Frequency	Percentage
Death	55	55	62	28.9
Discharge	45	45	153	71.2
	χ^2=20.01; p=0.001			

were also supported by systematic review of Horsting WB et al (2015). The authors in the reference study observed infections as the common cause in developing country but overall the most common causes for NTC were stroke, post-anoxic coma, poisoning and metabolic[9].

Our study also documented significant improvement in outcome of patients of phase 2 as compared to outcome of patients 15 years back. This could be explained by technological advancement and overall improvement in medical care of the patients.

CONCLUSION:

Our study provides a detailed description of change in etiology of non traumatic coma over the 15 years. We observed that the NTC was predominantly associated with infective causes 15 years back but in present era, non infective causes such as CVA is the leading cause of NTC. However, infective causes of NTC still persist. The understanding of shift of etiology from infective to non infective cause is essential to provide early diagnosis and appropriate

treatment of non traumatic coma. So it is strongly recommended that primordial and primary prevention should be the mainstay of intervention to reduce the burden of NCD on health care facility in the country.

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Non-Invasive Detection of Helicobacter Pylori Infection: A Comparative Study

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ABSTRACT

The present study aimed to determine the diagnostic accuracy of serological testing in endoscopic Rapid Urease Test(RUT) positive *H. pylori* infections. The study included 50 participants, above the age of 18 years, who attended Outpatient Department or were admitted in the tertiary a care hospital complaining dyspepsia and were positive for *H. pylori* on endoscopic RUT. Those patients who were found positive were subjected to serological testing for *H. pylori* infection by card test (Immunochromatography method). The results of RUT biopsy and serological testing were compared. Among the 50 patients enrolled in the study, 62% (n=31) were males while 38% (n=19) were females (Male to Female ratio 1.63:1). Majority of patients belonged to the age group, 31 to 60 years (50%, n=25). Sixty percent (n=30) of the patients belonged to upper-lower class according to the Kuppuswamy's scale. Out of the 50 patients who were positive for *H. pylori* on RUT assay, 84% (n=42) came out to be positive on Immunochromatography. Thus, keeping RUT assay as the reference test, the sensitivity of immunochromatography was found to be 84%. There was a significant association found between between age and the positivity of immunochromatography (p value 0.03). The ICT used in this study is commercially available, inexpensive, and easy to perform. Positive predictive value of 84% implies that the test could be used to identify *H. pylori* infections in patients with upper gastrointestinal symptoms. Attributed to the rapidity of test results, clinical decisions regarding patient care could be made during the visit.

KEY WORDS: helicobacter pylori, immunochromatography, infection, serological test

INTRODUCTION:

There have been numerous associations of Helibacter pylori with various human diseases including gastric ulcer and even malignancies[1]. Attributed to its cytotoxin-associated geneA (cagA), vacuolating toxin A (vacA) and adherence factors, this Gram-negative bacterium shows a strong predilection to gastric mucosa, thus leading to clinical manifestations such as dyspepsia[1]. Early detection and treatment of *H. pylori* infection is of utmost importance as it may otherwise lead to great morbidity and mortality in later stages. Various investigations can be used for detection of *H. pylori* infection. Endoscopy-based biopsy followed by biopsy urease test, histological examination and microbiological culture of the biopsy specimen have proven to be helpful diagnostic methods. However, non-invasive tests like serology, ^{13}C urea breath test and stool antigen test are simpler and inexpensive methods of *H. pylori* detection. Urea breath test has been a good screening tool for detection of *H. pylori* but it requires fasting. Stool antigen test being non-invasive is helpful as well but specimen collection can slightly delay the diagnosis. Various serological tests can also be used for the detection of *H. pylori*, via measurement of IgG, IgM and IgA antibodies in the serum. These have excellent sensitivity and specificity of about 95%. The exact role of serology in the management of *H. pylori* is still to be defined although there is evidence that using this as a screening procedure can reduce endoscopy cost and work overload[2]. Moreover, serological tests are the only tests which are not likely to give false negative results in patients who have taken antibiotics, bismuth compounds or omeprazole in the recent past[3].

MATERIALS AND METHODS:

This study was a prospective, observational study conducted among patients presenting with features of dyspepsia at People's College of Medical Sciences and Research Centre, Bhanpur, Bhopal.

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The study aimed to determine the diagnostic accuracy of serological testing in endoscopic RUT positive *H. pylori* infections. The study included 50 participants, above the age of 18 years, who reported to the Outpatient Department or were admitted in the hospital with dyspepsia and were positive for *H. pylori* on endoscopic RUT.

The study excluded patients negative for *H. pylori* on endoscopic Rapid urease test (RUT), chronic alcoholics, patients on long term NSAIDs/ proton pump inhibitors, patients with past history of *H. pylori* eradication or who have undergone upper gastrointestinal surgery.

After obtaining written, informed consent, detailed history was taken and each patient was assessed for symptoms of GERD. For assessing the patient's socioeconomic status, modified Kuppuswamy's socio-economic classification was used. Upper GI endoscopy was done to test the presence of *H. pylori* by obtaining biopsy followed by RUT. Those patients who were found positive were subjected to serological testing for *H. pylori* infection by card test (Immunochromatography method).All the *H. pylori* positive patients were administered eradication therapy for 14 days followed by acid suppression therapy for 6 weeks. The results of RUT biopsy and serological testing were compared and statistically analyzed using SPSS software ver. 19.0. p value <0.05 was considered as significant.

RESULTS:

Among the 50 patients enrolled in the study, 62% (n=31) were males, while 38% (n=19) were females depicting a male to female ratio of 1.63:1. Majority of patients belonged to the age group, 31 to 60 years (50%, n=25). Sixty percent (n=30) of the patients belonged to upper-lower class according to the Kuppuswamy's scale (Table 1).

Table 1: Study population according to socio economic status

Socio economic status	Number of patients	Percentage
Lower middle	13	26
Upper lower	30	60
Upper middle	7	14
Total	50	100

Out of the 50 patients who were positive for *H. pylori* on RUT assay, 84% (n=42) came out to be positive on Immunochromatography. Thus, keeping RUT assay as the reference test, the sensitivity of immunochromatography was found to be 84%.

Out of the 42 patients who were found positive for *H. pylori* in immunochromatography, 47.6% (n=20) patients belonged to the age group of 31-60years (Table 2). There was significant association found between age and the positivity of immunochromatography (p-value 0.03). No significant association was found among gender, educational status, socio-economic status and the results of positivity of immunochromatography (Table 3).

Table 2: Association between age group and immunochromatography (ICT) findings.

Age group	ICT negative	ICT positive
18 to 30 years	3 (37.5%)	12 (28.6%)
31 to 60 years	5 (62.5%)	20 (47.6%)
>60 years	0	10 (23.8%)

DISCUSSION:

Humans harboring *H. pylori* in their gastric mucosa develop serum antibodies to the organism. Currently, these antibodies can be detected by several methods, including ELISA we evaluated the accuracy of an immunochromatographic method to detect anti-H. pylori IgG in human serum. The ICT had a high sensitivity and specificity for detection of *H. pylori* IgG antibody in serum from randomly selected patients. Similar results have been reported by another study[4].

In the current study, majority of the patients were in the age group between 31-60 years (50%, n=25). Gill et al[8] (1993) from India reported most of the clinical suspects in the age group between 30-39 years. In this study, 62% (n=) were male while rest were female. Similar findings of male dominance (91%) was reported in another study by Morshed et al[9] (2008) from Bangladesh.

In this study, majority of the patients (60%) were from upper lower class and rest were from upper middle and lower middle class. The prevalence of infection is correlated with low socioeconomic status during childhood, high density of living and low household income. Poor hygiene and crowded conditions may facilitate transmission of infection among family members and is consistent with intrafamilial and institutional clustering of *H. pylori*

Table 3: Association between gender, educational status and socio-economic status with the findings of immunochromatography (ICT).

Group	ICT negative	ICT positive
Gender (p value 0.4)		
Females	2 (25%)	17 (40.5%)
Males	6 (75%)	25 (59.5%)
Educational status (p value 0.51)		
Graduate	2 (25%)	11 (26.2%)
Higher secondary	2 (25%)	3 (7.1%)
High school	1 (12.5%)	7 (16.7%)
Middle school	1 (12.5%)	3 (7.1%)
Primary school	0	9 (21.4%)
Illiterate	2 (25%)	9 (21.4%)
Socio-economic status (p value 0.42)		
Lower middle	2 (25%)	11 (26.2%)
Upper lower	6 (75%)	24 (57.1%)
Upper middle	0	7 (16.7%)

infection. In a study by Mahalanabis et al (1996) from Bangladesh reported high *H. pylori* infection rate in children, in poor community and explained an association with contaminated environment, crowding, lack of proper sanitation and lack of sufficiently clean water. These findings from previous study are not in agreement with our study because the population of patients visiting in our OPD are from upper and middle class. In our study, ICT was found positive in 84% of the study population which almost correlates with a study done by Andersen et al[10] (1996). They studied Danish population and found positive ICT results in 70.6% cases and suggested that ICT measurements in future serologic screening may improve diagnostic sensitivity considerably.

Average values of sensitivity and specificities of both tests were calculated in an overview of epidemiology and diagnosis of *H. pylori* infection by Logan and Walker. With the sensitivity of 84%, immunochromatography was within the range of average values. For serology, the sensitivities were 80-95% and specificities ranged between 80-95%.

Monotherapy with commonly used antibiotics, such as metronidazole or clarithromycin, can achieve H. pylori eradication rates in up to 17±20% patients. Serum antibody tests remain positive for a significant period, or perhaps indefinitely, after *H. pylori* eradication. Remote ingestion of certain antibiotics in potential study candidates could therefore result in eradication of *H. pylori*. Such patients would have negative tests for urease activity and (or) non visualization of bacteria on histology in presence of detectable serum antibodies (positive ICT). Such asituation would result in 'false-positive' antibody test results and could contribute to a lower specificity of the ICT. Other conditions that could potentially lower the specificity of antibody-based tests include recent antibiotics ingestion, bismuth compounds, and proton pump inhibitors. These agents alter gastric mucosal inflammation, bacterial distribution, and urease activity, making histologic detection of *H. pylori* more difficult and thereby increase the chances for false-negative *H. pylori* infection status[5,6,7,8]. The use of such products was and exclusion criteria for our study. Identification of *H. pylori* infection has become critical in the management of patients with gastroduodenal ulcer disease. The pathogenesis of *H. pylori*-related diseases is not clearly understood, but eradication of this infection favorably alters the natural history of gastroduodenal ulcer disease. Currently, serology plays an important role in two situations, i.e., screening of various populations to understand the epidemiology of *H. pylori* infection, and to identify those patients with gastroduodenal ulcer disease who are infected. Infected patients with active or quiescent gastroduodenal disease require antibiotic therapy. Absence of *H. pylori* infection, as manifested by negative serologic tests, in patients who are not exposed to non-steroidal anti-inflammatory drugs almost completely excludes the presence of gastroduodenal ulcer disease as well as the precursor lesions of gastric carcinoma[10-14].

CONCLUSION:

The ICT used in this study is commercially available, inexpensive, and easy to perform. Positive predictive value of 84% implies that the test could be

used to identify *H. pylori* infections in patients with upper gastrointestinal symptoms. Attributed to the rapidity of test results, clinical decisions regarding patient care can be made during the visit. Thus, antibody testing is expected to continue to improve and play an ever-increasing role in the primary clinician's diagnostic evaluation. As the *H. pylori* infection rate is known to be high in Indian population, recommendations regarding investigations with serological testing can be of great benefit in this population.

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Clinico-Histopathological Study of Primary Localised Cutaneous Amyloidosis with Histopathological Correlation by H & E and Congo Red Stain

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ABSTRACT

Primary localized cutaneous amyloidosis is a commonly encountered problem in our scenario. As there is paucity of Indian studies on this subject, a clinico-histopathological study is required. Moreover it resembles with other cutaneous hyperpigmented lesions so on the part of clinician it will be helpful to properly diagnose the condition. To determine various clinical variants of primary localised cutaneous amyloidosis and its clinico-histopathological correlation by H & E and Congo red staining. Study was undertaken on all new patients with primary localised cutaneous amyloidosis attending skin OPD of People's College of Medical Sciences and Research Centre (PCMS & RC). After detailed history and clinical examination punch biopsy of skin was taken for histopathological examination after written consent. Daily data collection was done in a pre designed proforma and was recorded and analyzed with appropriate statistical test. The most commonly encountered clinical variant in present study was Macular amyloidosis followed by Lichen amyloidosis. Present study shows, it is difficult to diagnosed biphasic variant clinically, however histopathology can differentiate it where feature of both macular and lichen variants are present. Congo red stain under polarized light was found to be superior in confirming the diagnosis than H&E stain on light microscopy.

KEY WORDS: congo red, cutaneous amyloidosis, H&E

INTRODUCTION:

Amyloid term was introduced by "Rudolf Virchow", who described these extracellular precipitates that turn brown when they are incubated with iodine[1]. Amyloidosis has been defined as deposition of abnormal extracellular fibrillar proteinaceous material in tissues and is related to the family of proteins that are biochemically unrelated and has certain characteristic staining properties like apple green birefringence when they are stained with Congo red and viewed under polarized light. Broadly these deposits can be classified in Systemic amyloidosis where there is deposition throughout many organs of the body and Localized amyloidosis where it is limited to a single tissue site or organ[1].

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MATERIALS AND METHODS:

The study was conducted in Dermatology outpatient department, People's College of Medical Science and Research Centre and associated People's Hospital Bhopal. The study enrolled 55 patients with PLCA fulfilling the inclusion criteria and exclusion criteria noted below over the period of 1 year & 4 months i.e. from 01/01/18 to 30/04/19. Patients willing to participate, new patients of all age groups and both sexes with a clinical diagnosis of PLCA, patients diagnosed with all clinical types of PLCA i.e. Macular, Lichen and Biphasic amyloidosis were included in the study.

Pregnant women, patients with features of systemic amyloidosis, already diagnosed cases of PLCA on follow up or under treatment, patients not willing to participate were excluded from the study. Informed consent was obtained after informing the study subjects the details of the procedure. After detailed history and clinical examination all the cases diagnosed clinically were then subjected to punch biopsy of skin for histopathological examination by H&E stain and then further confirmed by special stain

i.e. Congo red stain. Daily data collection was done in a predesigned proforma and was recorded. The data was compiled and analysed with "spss version 20" software.

RESULTS:

Mean age of patients in present study was 36.04 ± 12.31 years. Maximum patients (34.5%) belonged to 31 to 40 years of age followed by 27.3% in the age range of 21 to 30 years. Only 9.1% patients were less than 20 years of age. Majority of patients with PLCA were females (74.5%) whereas male constituted 25.5% of the study population. In present study, 45.5% patients were housewives followed by students (16.4%) and office worker (12.7%). Only 7.3% patients were laborer and government employee each. Duration of disease in majority of patients was 1 to 5 years (58.2%) and the mean duration of disease for all the patients was 5.71±5.03 years. Only 9.1% patients presented with duration less than 1 year. Upper limb and back were the most common sites involved in 87.3% and 80% patients respectively. Least commonly involved site was chest and abdomen observed in 7.3% patients. History of friction/scrub in cases of PLCA was documented in 52.7% patients in present study whereas it was absent in 47.3% patients. Pruritis was observe d in 80% cases. PMLE was the most common associated condition observed in 9.1% followed by hypothyroidism in 7.3% cases. Diabetes, hypertension and history of atopy were associated conditions in 5.5% patients each. Family history of PLCA was observed in 10.9% cases. Most common clinical diagnosis was macular in 90.9% cases whereas lichen amyloidosis was diagnosed clinically in 9.1% cases. None of the cases was diagnosed as biphasic amyloidosis clinically. Most common pattern of PLCA was rippled observed in 78.2% cases followed by confluent pattern and papules which was seen in 12.7% and 7.3% cases respectively. However 1.8% cases were mixed pattern i.e. papules with plaques.

H&E staining under light microscopy was suggestive of macular amyloidosis in 40% cases whereas it was suggestive of lichen and biphasic amyloidosis in 5.5% cases each. Congo red was suggestive of macular amyloidosis in 74.5% cases, lichen amyloidosis in 9.1% cases and biphasic amyloidosis in 7.3% cases (Figure 1). 90.9% patients were identified as positive by Congo red stain under polarized light whereas only 50.9% cases were detected by H&E stain. The observed difference in the diagnosis by two method was statistically highly significant (p = 0.001) (Table 1). Out of 55 cases of PLCA, 4 cases were diagnosed as negative by both the diagnostic modalities and thus they were considered true negatives (Table 2). Congo red has 98.03% sensitivity whereas sensitivity of H & E was only 54.9%. Similarly, NPV was 80% for Congo red whereas it was only 14.8% for H & E. However, specificity as well as PPV was 100% for both the tests (Figure 2).

Table 1: Distribution according to findings of H&E and Congo red stain.

	Congo red	H & E	Chi sq	p-value
Positive	50 (90.9)	28 (50.9)	21.33	0.001
Negative	5 (9.1)	27 (49.1)		

Table 2: Diagnostic accuracy of H & E and Congo red stain.

Test	PLCA	
	Present	Absent
Congo red stain		
Positive	50 (TP)	0 (FP)
Negative	1 (FN)	4 (TN)
H & E stain		
Positive	28 (TP)	0 (FP)
Negative	23 (FN)	4 (TN)

DISCUSSION :

Cutaneous amyloidosis is a type of organ-specific amyloidosis which can be primary localized (PLCA) or secondary cutaneous amyloidosis[3]. Uncommon variants are dyschromic amyloidosis and familial amyloidosis[7]. Amyloid deposits are characterized by amorphous, eosinophilic, acellular material on routine hematoxylin and eosin (H&E) staining and the congo-red stain gives an orange-red staining reaction to these deposits on light microscopy, which show apple-green birefringence when visualized under polarized light. The staining characteristics are due to the cross-beta-pleated sheet conformation of the polypeptide backbones of the amyloid fibrils. These fibrils are ultra structurally 8 to 12 nm in width and of indeterminate length. Chemically, there are more than 16 different amyloids[8].

In present study, most common clinical diagnosis was macular in 90.9% cases whereas lichen amyloidosis was diagnosed clinically in 9.1% cases. None of the cases was diagnosed as biphasic amyloidosis clinically. Kilaparty K et al (2016) also observed macular amyloidosis as the most common type of PLCA[9]. George AE et al (2017) diagnosed 20 cases as lichen, 11 as macular and only 7 as biphasic
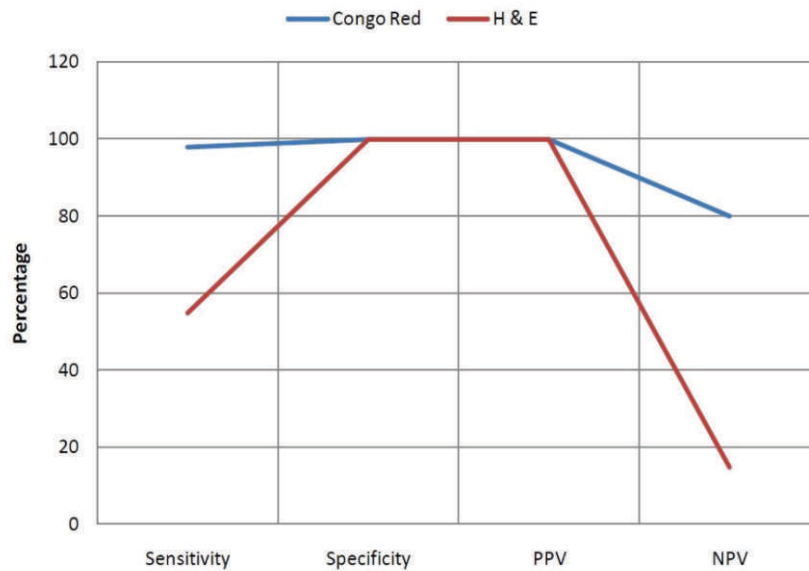
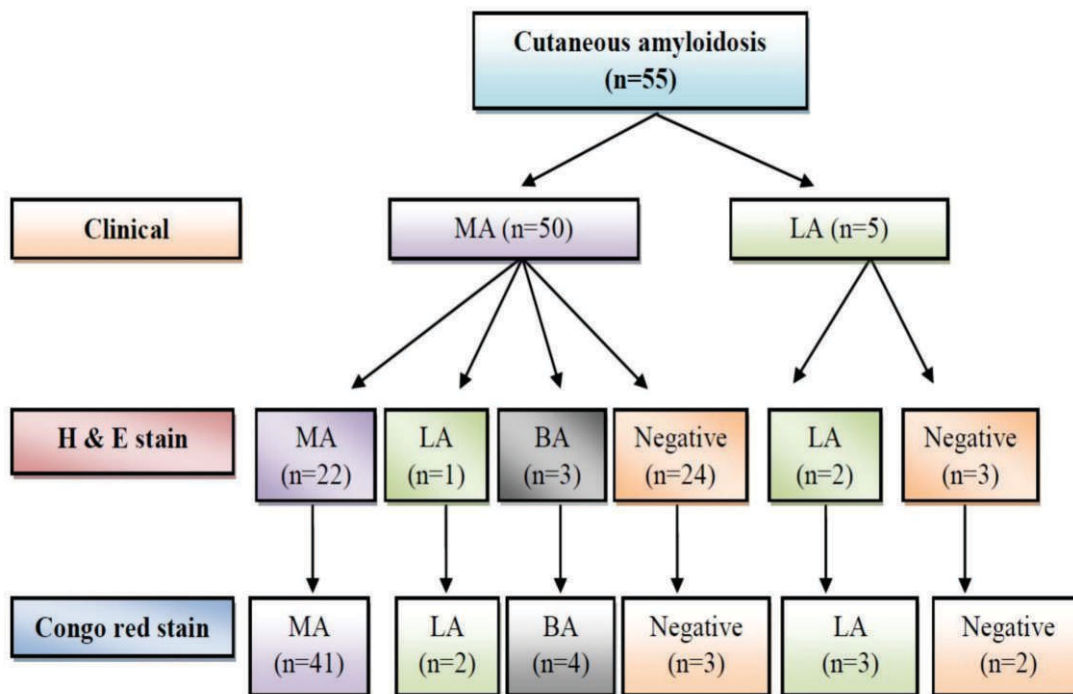**Figure 1:** Diagnostic pattern of cutaneous amyloidosis.

Figure 2: Diagnostic Accuracy of H&E and Congo Red.

*MA: Macular Amyloidosis; *BA: Biphasic Amyloidosis; *LA: Lichen Amyloidosis

amyloidosis clinically.[7] Thus most common amyloidosis was macular in present study whereas it was lichen amyloidosis in reference study. Mehrotra K et al (2017) in their review described three variants of PLCA as lichen, macular, and biphasic amyloidosis. Of them, macular was most common followed by lichen amyloidosis[10].

In present study, out of 55 clinically diagnosed cases of PLCA, H&E staining was suggestive of macular amyloidosis, lichen amyloidosis and biphasic amyloidosis in 40%, 5.5% and 5.5% patients respectively. Similarly, Congo red was suggestive of macular amyloidosis in 74.5% cases, lichen amyloidosis in 9.1% cases and biphasic amyloidosis in

7.3% cases. However, it was negative in 9.1% cases. Congo red positivity was observed in 90.9% cases whereas H&E positivity was observed in only 50.9% cases in present study. Sensitivity of Congo red and H&E was 98.03% and 54.9% respectively. However, NPV was 80% for Congo red whereas it was only 14.8% for H&E. The present study documented specificity as well as PPV as 100% for both the tests. The findings of present study were in concordance with Panicker Vet al (2017), in which Congo red positivity was observed in 53.8% with a sensitivity of 85%, however the authors concluded that Congo red stain under immunofluorescence microscopy is more sensitive than polarizing microscopy[11].

Vijaya B et al (2012) documented 100% sensitivity of Congo red in their study. They concluded that though, H&E stain gives a clue for the diagnosis of amyloid nevertheless Congo red staining under polarized light forms a very sensitive and definitive method for confirmation[12].

CONCLUSION:

The most commonly encountered clinical variant in present study was Macular amyloidosis followed by Lichen amyloidosis. Present study shows, it is difficult to diagnosed biphasic variant clinically, however histopathology can differentiate it where feature of both macular and lichen variants are present. Congo red stain under polarized light was found to be superior in confirming the diagnosis than H&E stain on light microscopy.

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Role of Intralesional Vitamin D3 in the Treatment of Cutaneous Warts

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ABSTRACT

Warts are the benign cutaneous manifestations caused by human papillomavirus (HPV). All currently available treatment modalities are associated with significant side effects and frequent recurrences. As intralesional vitamin D3 is a new immunotherapeutic agent in practice, we conducted this study to determine its safety and efficacy as an immunotherapeutic agent in the treatment of cutaneous warts. We conducted this study on 60 patients. The selected warts were slowly injected with 0.1-0.5 ml of Vitamin D3 (15 mg/ml) into the base of each wart with a 26-gauge insulin syringe. A maximum of 5 warts were injected per session. The injections were performed at 2 weekly intervals until complete resolution or for a total of 4 sessions. It resulted in complete clearance in 38 out of 60 patients, while moderate response was seen in 4 patients and mild response was observed in 3 patients in terms of number of warts. Fifteen patients did not demonstrate any decrease in number of warts even after 4 sittings of intralesional Vitamin D3. In terms of size of warts, moderate response was seen in 8 patients and mild response was observed in 5 patients. Nine patients did not demonstrate any reduction in size of warts even after 4 sittings of intralesional Vitamin D3. Immunotherapy with vitamin D3 seems to be a promising, effective, simple, inexpensive and safe treatment modality for the treatment of cutaneous warts. Due to low recurrence rates, it can be considered to have potential advantages of widespread and sustained effects against HPV.

KEYWORDS: intralesional immunotherapy, vitamin D3, warts

INTRODUCTION:

Warts are the benign cutaneous manifestations caused by human papillomavirus (HPV)[1]. Most patients seek treatment of warts since they are cosmetically disfiguring and sometimes painful, especially on the soles[2,3]. Local destruction of warts is a commonly employed treatment modality performed by using either electrocoagulation, cryotherapy, laser therapy or by topical keratolytics like salicylic acid and trichloroacetic acid[4]. All these treatment options can be painful and may be associated with scarring and frequent recurrences. In addition, destructive modalities are not suitable for the treatment of multiple warts as they clear only treated lesions and have no effect on the distant ones[5,6].

Various agents have been tried for intralesional immunotherapy including measles, mumps, rubella vaccine (MMR), Bleomycin, Tuberculin purified protein derivative (PPD),

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Bacillus-Calmette-Guerin (BCG) vaccine, Mycobacterium w vaccine and Candida antigen. Other imunotherapy agents include *Corynebacterium parvum*, contact immunotherapy, glycyrrhizinic acid, Echinacea, green tea extracts, and intralesional vitamin D with variable results. Among these, contact immunotherapy with dinitrochlorobenzene (DNCB), diphencyprone and squaric acid dibutyl ester (SADBE) have also been used but their use was limited by adverse effects like allergic contact dermatitis, urticaria and pigmentary disturbances[7,8,9,10].

In order to improve the outcome, intralesional immunotherapy is being tried widely for the treatment of multiple cutaneous warts over the last few years. It acts on the basic principle of enhancing the cell-mediated immunity against HPV and results in clearance of warts[11]. The role of vitamin D3 in the treatment of warts is still not well understood. The probable mechanism of vitamin D3 in the treatment of warts was proposed to be due to its ability to regulate epidermal cell proliferation and differentiation and modulate cytokine production. It can also lead to induction of anti-microbial peptide expression in the skin[3,12].

Intralesional immunotherapy reportedly causes the resolution of these longstanding benign

proliferations at the primary as well as distant sites. The exact mechanism of immunotherapy has not been completely elucidated but is believed that the injection to the HPV-infected tissue induces a strong nonspecific pro-inflammatory signal and attracts the antigen-presenting cells. This is associated with the release of different cytokines such as IL-2, IL-8, IL-12, IL-I8, tumor necrosis factor–α, and interferon-γ. Significant peripheral mononuclear cell proliferation promotes a Th1 cytokine response. This successively activates the cytotoxic T cells and natural killer cells to eradicate the HPV-infected cells[2,9,13,14].

As intralesional vitamin D3 is a new immunotherapeutic agent in practice, there is paucity of literature regarding its effectiveness. Therefore, we conducted this study to determine the safety and efficacy of vitamin D3 as an immunotherapeutic agent in the treatment of cutaneous warts.

MATERIALS AND METHODS:

The study was conducted on 60 patients over a period of 18 months. The study started after obtaining approval from the Research Advisory Committee (RAC) and the Institutional Ethics Committee (IEC) of People's College of Medical Sciences & Research Centre, Bhopal. (Code number IEC-2017/37).

The procedure was performed on all adult patients presenting to dermatology OPD with cutaneous warts, which were either newly diagnosed or left untreated for the past 6 months. Written Informed consent was obtained from all the patients after detailed explanation of the procedure prior to commencement of treatment.

The characteristics of the warts such as size and number of warts, type of warts, presence or absence of side effects, and clinical photographs were recorded at the start of the study and at each follow-up visit.

Vitamin D3 for injection, available in vials containing 6,00,000 IU of cholecalciferol in 1 ml (15 mg) was used to treat the patients. The selected warts were slowly injected with 0.1-0.5 ml of Vitamin D3 (15 mg/ml) into the base of each wart with a 26-gauge insulin syringe. Post-treatment, the patients were advised not to use any topical and oral medications. A maximum of 5 warts were injected per session. The injections were performed at 2 weekly intervals until complete resolution or for a total of 4 sessions.

If complete clearance was achieved before four injections, the treatment was stopped and all patients were followed up for two months after complete clearance to check for recurrence. Clinical response was documented by recording the decrease in number and size of warty lesions at each visit i.e., at 2 weekly intervals for 4 sessions. Clearance was considered in terms of reduction in both size and number of lesions. Response for further categorized accordingly: Complete response: 100%; Moderate response: between 50 to 99% and Mild response: between 1% to 49%. Data was compiled using MS Excel and statistically analyzed using SPSS/PC (Statistical Package of social sciences for personal computer) software version 20.

RESULTS:

Most common type of cutaneous wart observed in present study was palmoplantar (63.3%) followed by mixed warts in 13.3%. Common and flat warts were observed in 6.7% patients each. Mosaic type was the least common type of cutaneous wart observed in only 1.7% patients.

Mean number of injections administered in present study were 2.92±1.03 (range-1 to 4). About 38.3% patients required 4 sittings which also includes those patients who had partial clearance as well as no response. (Table 1).

In present study, 100% resolution in warts was seen in 63.3% patients following intralesional injection of vitamin D3 whereas no resolution was observed in 25% patients. More than 50% and less than 50% resolution in number of warts was observed in 6.7% and 5% patients respectively.

Complete resolution in size of warts was observed in 63.3% patients. However no resolution in size was observed in 15% patients following intralesional injection of vitamin D3. More than 50% reduction in size was observed in 13.3% patients whereas less than 8.3% reduction was observed in 8.3% patients only. In present study, we observed statistically highly significant reduction in number as well as size of warts at final follow up following intralesional vitamin D3 (p<0.01) (Table 2). The most common side effect observed in present study was pain during injection (100%) followed by hypopigmentation (6.7%), granuloma and swelling in 3.3% patients each. Secondary infection was observed in only 1.7% patients (Table 3). Recurrence of cutaneous warts was observed in only 2 (5.3%) patients following vitamin D3 injection. In present study, no significant association was observed between percentage reduction in number of warts and pattern of warts (p>0.05).

Table 1: Number of Injections administered at 2 weeks interval

Number of injections	Frequency (n=60)	Percentage	Outcome
1	6	10.0	Complete clearance
2	16	26.7	Complete clearance
3	15	25.0	Complete clearance
4	23	38.3	Complete + Partial + No clearance

Table 2: Reduction in mean number and size of warts.

Warts	At presentation		At final follow up		t- test	p-value
	Mean	SD	Mean	SD		
Number	5.13	8.02	1.58	3.74	3.98	0.001
Size	3.14	1.99	0.65	1.12	9.46	0.001

Table 3: Distribution according to side effects.

Side effects	Frequency	Percentage
Pain during injection	60	100
Hypopigmentation	4	6.7
Granuloma	2	3.3
Swelling	2	3.3
Secondary Infection	1	1.7

Figure 1: pre-treatment photograph showing plantar warts over left great toe (patient 1).

Figure 2: Post-treatment photograph showing complete resolution (patient 1).

DISCUSSION:

The effect of vitamin D3 derivatives on verruca is speculated from its potential to regulate epidermal cell proliferation and differentiation and to modulate cytokine production. Labaindera J et al (2005) suggested that toll-like receptor (TLR) activation of human macrophages upregulated the expression of vitamin D receptor and vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide. Thus suggesting an association of TLRs and vitamin D-mediated innate immunity and their possible role in antiviral efficacy[15].

In our study all the 60 patients presenting with cutaneous warts were given intralesional Vitamin D3 injections. Injections were repeated at 2 weekly intervals for a maximum of 4 injections and resolution was noted. If complete resolution was obtained before completion of 4 injections, the treatment was stopped. Mean number of injections required in present study were 2.92±1.03 (range-1 to 4) which were less as compared to study by Raghukumar S et al (2017)[16]and Kavya M et al (2017)[3].

Most common type of cutaneous wart observed in present study was palmoplantar followed

Figure 3: Pre-treatment photograph showing plantar warts over right first toe cleft space (patient 2).

Figure 4: Post-treatment photograph showing complete resolution (patient 2).

by mixed warts. These findings were similar to study by Kavya M et al (2017)[3] and Naresh M et al (2019)[2].

Mean number and size of warts at presentation and that following intralesional vitamin D3 injection at final follow up showed a difference that was statistically highly significant (p<0.01). We observed complete resolution in size as well as number of warts in 63.3% patients following intralesional vitamin D3 injection.

The complete resolution observed in present study was somewhat less as compared to complete resolution reported in 80% patients by Aktas H et al (2016) [17]. Kavya M et al (2017) observed complete resolution of warts in 78.6% cases similar to present

study[3]. Akula ML et al (2018) also reported complete resolution of warts in 70% patients following intralesional vitamin D3 injections[18].

Minimal side effects were observed in our study, the most common being pain during injection followed by hypopigmentation, granuloma, swelling and secondary infection. Singh SK et al (2018) also documented only pain at the site of injection[19]. Our findings were similar to findings of Banoth S et al (2019) documented like pain, redness and swelling at the site of injection[20].

Recurrence of cutaneous warts was observed in only 2 patients. Our findings were in concordance to findings of Kavya M et al (2017) and Naresh M et al (2019) who reported recurrence in 1 and 4 cases respectively.

Immunotherapy with vitamin D3 seems to be a promising, effective, simple, inexpensive and safe treatment modality for the treatment of cutaneous warts. Due to low recurrence rates, it can be considered to have potential advantages of widespread and sustained effects against HPV.

So it may be considered as a first line therapy for multiple warts and a second line therapy for warts recalcitrant to standard treatment modalities because of its low cost, good tolerability and widespread effect involving both treated as well as untreated warts and low recurrence rates.

CONCLUSION:

Immunotherapy with vitamin D3 seems to be a promising, effective, simple, inexpensive and safe treatment modality for the treatment of cutaneous warts. Due to low recurrence rates, it can be considered to have potential advantages of widespread and sustained effects against HPV. So it may be considered as a first line therapy for multiple warts and a second line therapy for warts recalcitrant to standard treatment modalities because of its low cost, good tolerability and widespread effect involving both treated as well as untreated warts and low recurrence rates.

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Compound Odontome: A Case Report

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ABSTRACT

One of the most common odontogenic tumors are odontomes. The term refers to tumors which are of odontogenic origin. Odontomes are basically of two types; compound odontomes and complex odontomes. Mostly they are asymptomatic. Presenting a case report of an odontome in a 10 year old female child in maxillary left anterior tooth region.

KEY WORDS: compound odontome, impacted lateral incisor, odontogenic tumour, surgical excision

INTRODUCTION:

The term *odontome* was coined in 1867 by Broca. Paul Broca defined odontome as "tumors formed by the overgrowth or transitory of complete dental tissues."[1] WHO also defined compound odontome as "A malformation in which all dental tissues are represented in a more orderly pattern than in the complex odontome, so that the lesion contains many tooth like structures. Most of these structures do not morphologically resemble the teeth in the normal dentition; however enamel dentin cementum and pulp are arranged as in the normal tooth."[2] Of all the odontogenic tumours, Odontomes are found to be in the range of 20-22%[3]. The occurrence of compound odontome is between 9-37% and the incidence is between 5-30%[4]. The predilection of occurrence of odontome is slightly higher in males (59%) than in females (41%)[5]. Here we present a case report of compound odontome in a 10 year old female patient in her maxillary left lateral incisor and canine region.

CASE REPORT:

A 10 year old patient reported to the Department of Pediatric and Preventive Dentistry with a chief complaint of painless swelling in the maxillary left lateral incisor region (Figure 1). The swelling was hard in consistency and without any signs of inflammation on the overlying soft tissue. Also the

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swelling was consistent in size. The orthopantomogram and the occlusal radiograph revealed multiple radio opaque tooth-like structures. The swelling was measuring10 mm mesiodistally and 6 mm vertically (Figure 2 & 3). After thorough examination of the hard swelling, surgical removal of the lesion/ overgrowth was planned. Blood investigation was advised before the surgery. Antibiotics and analgesics were prescribed for 5 days. Written consent was taken by the parents of the patient. The patient was recalled after 2 days for surgery under local anesthesia. After administration of local aneasthesia, the periosteal flap was raised using the Modified Widman Flap technique (Figure 4). After raising the flap, the overlying periosteum was remove using a micromotor borne cutting bur and irrigation was continuously carried out during the process of bone cutting to avoid production of heat. During the procedure it was observed that the hard tissue was attached with the periosteum from the labial side, however it was unattached from the palatal aspect. Hence it was easy to detach it from the palatal side. When the hard tissue lesion became completely free from the attachments, it was elevated and removed with the help of a periosteal elevator (Figure 5 & 6). After removal of the hard tissue, suture was given and patient was recalled after 1 week for suture removal (Figure 7). The specimen was sent for histopathological examination. The specimen was subjected to decalcification using 5% nitric acid. A portion of the specimen was studied by preparing a ground section. On microscopic examination of H and E stain section, normal enamel spaces, dentin and pulp tissue were observed which exhibited a normal relation to one another. Cementum presence was not evident. Thus it was confirmed that the hard tissue lesion was a compound Odontome. Follow up was

Figure 1: Hard swelling in left maxillary incisor region.
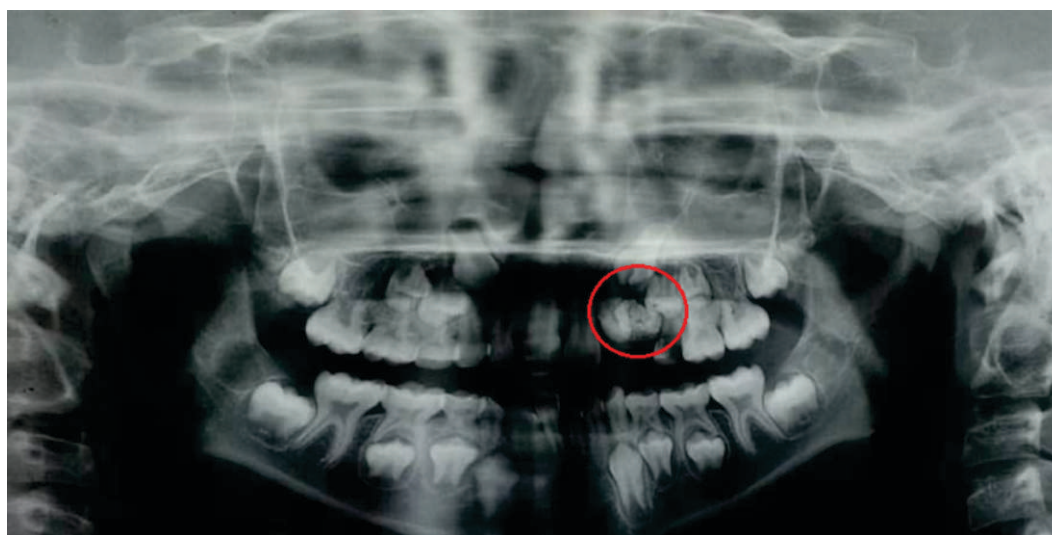

Figure 2: OPG showing multiple radio-opacities

Figure 3: Occlusal Radiograph

Figure 4: Exposed swelling after the flap.

Figure 5: Exposed odontome.

Figure 6: Excised hard tissue.

Figure 7: Application of suture

Figure 8: Follow up after 6 months.

Figure 9: IOPA of follow up after 6 months.

done after 6 months. Intra oral examination revealed eruption of 21 (Figure 8 and 9).

DISCUSSION:

Odontomes are usually seen in the first and second decades of life, and are vaguely accepted as being more of a developmental anomaly (hamartoma) rather than a true neoplasm[6]. Although the exact etiology of Odontome remains unknown, however various probable etiological factors have been proposed. Satish V *et al* proposed local causes, infection, mature ameloblasts, cell rests of serres, extraneous odontogenic epithelial cells, and trauma as the probable etiological factors[7]. Sometimes there are inadequate restricted spaces which creates growth pressures which result in the formation of odontomes. It was also proposed that pyogenic infection caused by treponema palladium during the stage of tooth development causes division of tooth germ which can

result in formation of odontomes. Mature ameloblasts are specialized cells that have the potential to develop tumors. These cells can also result in the formation of Odontomes. Cell rests of serres (also known as dental lamina remnants) of the retained tooth with some epithelial bands undergo proliferation which develop into odontomes. Previous history of any trauma can also result in the formation of a hard tissue odontome. A study by Syed MR *et al* has revealed that the incidence of odontome is more common in the maxillary arch(67%) than in the mandibular arch (33%) with a marked predilection for the incidence in the anterior region[8]. In this present case, there was a compound Odontome which was painless in nature. The only treatment option in this case according to the available literature was the surgical extraction of the odontome with complete removal of any associated soft tissue which was successfully performed. Further orthodontic treatment will be required for the correction of the malocclusion. Also, in our case we extracted the odontome via the conventional surgical method. But as per newer literature, surgical extraction of the odontome using Lasers is a recent advancement in the management protocol of odontomes. Angiero F *et al*[9] presented a retrospective study of recent clinical experience using Lasers for the surgical treatment of lesions of this kind and scored postsurgical pain. They concluded that a laser surgery reduces pain, having an excellent clinical outcome and minimizes treatment time.

CONCLUSION:

Early diagnosis and treatment can result in better occlusion and aesthetics.

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Large Benign Ovarian Cyst with Sub Acute Intestinal Obstruction

Case Report

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ABSTRACT

Sub acute intestinal obstruction is one of the most common problem faced by surgeons all over the world. However its association with ovarian tumor in rarely seen. It is more commonly seen with adhesions and neoplasm. Despite technological advances in diagnostic techniques, the diagnosis could only be made on surgery. Here is one such case where female presented with symptoms mainly mimicking pressure symptoms of ovarian cyst ,but on surgery sub acute intestinal obstruction was found.

KEY WORDS: benign, ovarian cyst, torsion, SAIO

INTRODUCTION:

Ovarian mass are often cystic, and are a common problem met in Gynaecological OPD[1]. Large masses usually present as abdominal distention, pain or discomfortdepending on location, size anddegree of compression. Acute abdomino- pelvic pain incidence comprised about 1.5% of OPD visits, and 5% of IPDs. Acute presentation may be in the form of severe pain and vomiting, and may be due to torsion of the ovarian mass or cyst. Rarely, they may present with sub-acute intestinal obstruction.

CASE REPORT:

A 35 yr old lady presented with chief complain of progressive abdominal distension since 1 year. There was generalized pain in abdomen off and on, since 3-4 days, and constipation since 1 week. It was also associated with vomiting off and on since 2-3 days which was projectile.

Her menstrual cycles were regular, and there was no history of overdue. Her Obstetric history is P6L4A2 with previous 2 LSCS and CTT done 4 years ago. There was a history of laparotomy done for ovarian cyst 5 years back.

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On general examination, patient was Ambulatory, conscious and oriented. Her Pulse was 90 bpm, and BP was 124/90 mmhg. On examining per abdomen, a firm non-tender mass of 30-32 weeks size with restricted mobility was palpable, fluid thrill was present. On Per vaginum examination, uterus was anteverted, multiparous size, mobile, and felt separated from the mass; movements were not transmitted to mass. Bilateral fornices was free, and mass could not be felt in either fornix or posterior fornix.

Transvaginal sonography showed large cystic lesion in right adnexa extending up to epigastric possibly of ovarian cyst, and minimal right side hydronephrosis. On CECT, there was alarge peripherally enhancing cystic lesion arising from right side of pelvis extending up to epigastric region - ? *Ovarian origin* probably benign. Serum CA-125 was within normal limits. All other laboratory parameter were within normal limit.

Patient underwent surgery and Exploratory Laparotomywith Omental biopsy was done. A huge twisted Left ovarian cyst of 20x17cm (3 twists) along with swollen fallopian tube (swollen and dark congested)was found . Mesentery was pulled up with twisted and distended recto sigmoid and descending colon. Twist of the bowel was about 180 degree which could have lead to volvulus. Exploration was done .Peritoneal surfaces and liver surface was smooth. Ascitic fluid of around 500ml was found, which was straw colored, and sent for cytology.Left salpingo-oophorectomy was done. Right ovary and tube was absent. Uterus was left behind as bladder was plastered
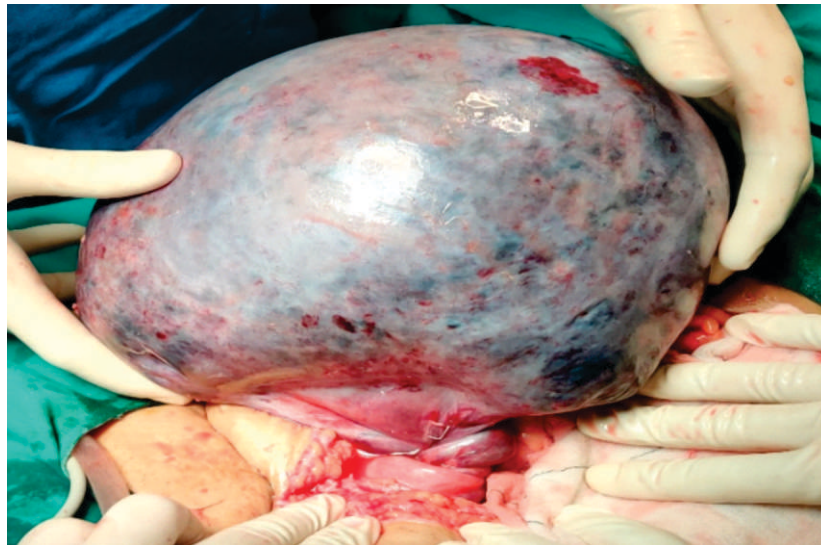
Figure 1: Huge hemorrhagic ovarian cyst, with 3 twists including intestine and mesentery.
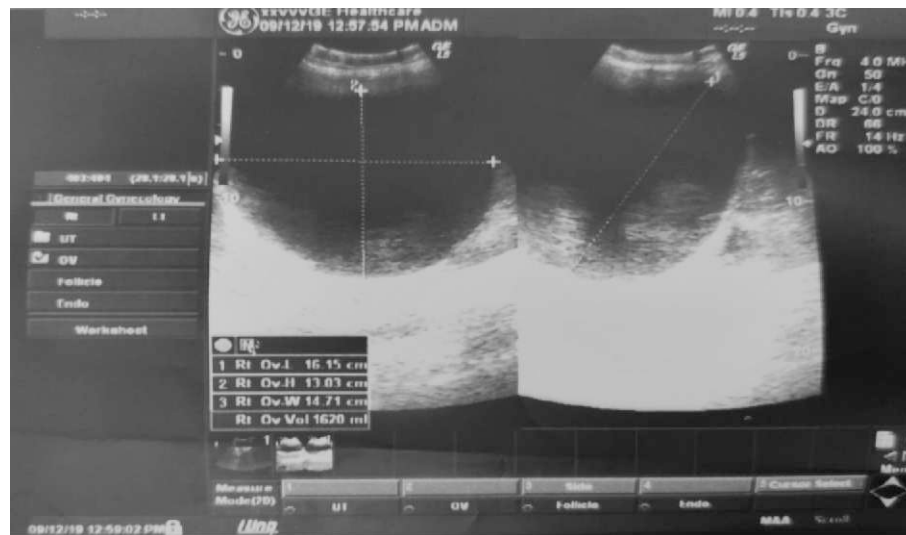

Figure 2: USG showing large cystic ovarian cyst.

to anterior surface of uterus, and was densely adherent. Omental biopsy was taken.

On Cut section of ovarian cyst, uniloculated, smooth lining,and filled with haemorrhagic serous fluid.Sample was sent for Histopathology examination which was evident of Haemorrhagic benign ovarian cyst with ovarin endometriosis. Patient stood procedure well. Her post-op period was uneventful.

DISCUSSION:

Sub acute intestinal obstruction is a rare presentation of ovarian tumors, but is a deadly presentation. Interestingly, ability of ovary to form cyst doesn't end with menopause. In the elderly, it may be seen with ovarian cancer. It is usually due to adhesions, and may be also be related to tumor growth and blockage[2]. It is frequently present in association with pleural effusion and ascites. In the neonatal period, the incidence of ovarian cysts complicated with intestinal obstruction is 3%, but is seldom seen in adult age group[3]. Torsion is less commonly observed on left side due to recto-sigmoid[4]. Diagnosis is difficult, and in majority of cases intra-operatively diagnosis is made. Delay in diagnosis, associated co-morbidities and advancing age are the reasons for high mortality .

CONCLUSION:

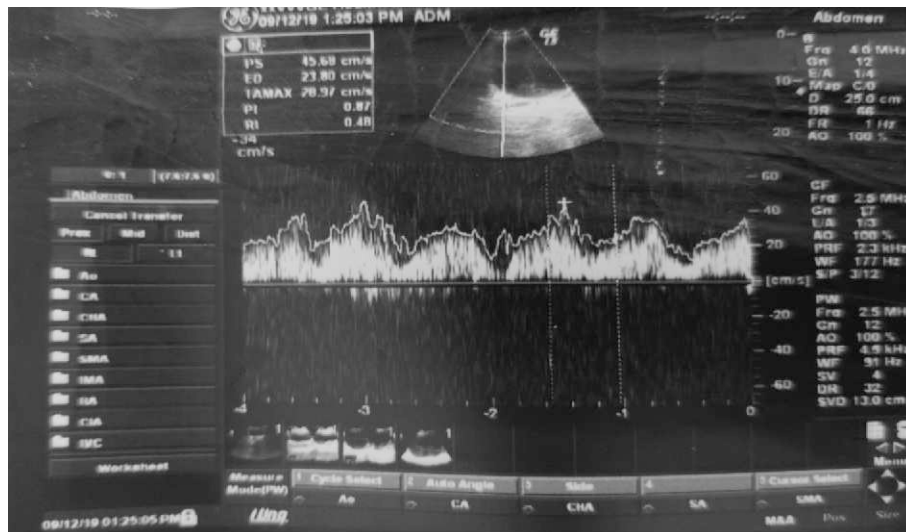Torsion in ovarian tumors may not always

Figure 3 : USG Color Doppler showed maintained vascularity.

present with dramatic symptoms and signs, and may present as SAIO. In the present case, the features of SAIO was because of trapping and twisting of bowel mesentery with the ovarian torsion. Delay in management as it presents with unusual symptom, may be dangerous, and hence emergent intervention must be done.

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